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Temporal Variation of Meristic Traits within an Atlantic Salmon (*Salmo salar*) Stock, and Implications for Stock Identification

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The assumption is generally made in stock identification studies that variation within stocks is not confounded with variation among them. To evaluate whether this assumption is tenable we test the null hypothesis of temporal stability in five key meristic traits of a single stock of Atlantic salmon (*Salmo salar*). We used smolts collected over a 10-yr period in the downstream migrations from one small river system. Individuals were tagged when captured and preserved, so we can estimate variability within, as well as among, runs. The meristic traits are remarkably homogenous within runs and cohorts. However, the null hypothesis of stability over time among runs and cohorts is strongly rejected for each trait. We conclude that temporal variation is both extensive and persistent. There is limited covariation among the traits, but they are largely independent of scores of environmental variables and of variation in the biological attributes of the stock. Because among-group variability is meaningful only in context of within-group variation, and because the within-group variation is large, our results have serious implications for sampling design in stock identification studies.

On considère généralement dans les travaux d'identification des stocks que la variation au sein d'un stock est différente des variations entre les stocks. Pour évaluer la solidité de cette approche, nous testons l'hypothèse nulle de la stabilité temporelle sur cinq traits méristiques dans un seul stock de saumons de l'Atlantique (*Salmo salar*). Nous avons prélevé des smolts sur une période de dix ans pendant la migration d'avalaison dans un petit système hydrographique. Les spécimens ont été marqués à la capture et conservés, de sorte que nous pouvons mesurer la variabilité dans une remonte et d'une remonte à l'autre. Les traits méristiques sont remarquablement homogènes à l'intérieur des remontes et des cohortes. Cependant, l'hypothèse nulle de la stabilité sur une période donnée parmi les remontes et les cohortes est fortement rejetée pour chaque trait. Nous concluons que la variation temporelle est à la fois générale et persistante. On note une covariation limitée entre les traits, mais ceux-ci sont nettement indépendants des résultats des variables environnementales et de la variation dans les attributs biologiques du stock. Étant donné que la variabilité intergroupes n'a de sens que dans le contexte de la variation intragroupe, et étant donné l'importance de cette variation intragroupe, nos résultats auront des incidences notables sur la conception des échantillonnages dans les travaux d'identification des stocks.

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That fish occur in "stocks" has become a basic tenet of fishery biology, and a cornerstone in the management of commercial and sport fisheries. The stock concept (Booke 1981; MacLean and Evans 1981; McDonald 1981) addresses the realities that individuals that are found together at a given time may derive from distinct breeding populations, and that each contributing population (stock) may have different biological characteristics. If individual stock size or recruitment is missestimated and a fishery continues harvesting at high levels, the more vulnerable (less productive) stocks may be seriously depleted or eliminated. Hence, in order to achieve optimal management of available resources, individual stocks must be treated as discrete management units.

While the stock concept is attractive, its implementation is not without difficulty. This is partly because differences between the constituent stocks within a mixed fishery may be subtle, making their identification and enumeration difficult. Consequently much effort is expended on the development of increasingly sophisticated techniques for stock identification (e.g. Fournier et al. 1984; Kenchington 1986; Misra 1985; Misra and Ni 1983; Winans 1984).

Different stocks generally have different geographic origins, so heavy use is made of traits that vary geographically. However, geographically variable traits may also vary temporally, and this temporal component of variation has been largely

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neglected in stock identification methodology. The assumption is generally made that temporal variation within stocks is not confounded with geographic variation among stocks or, more precisely, that within-stock variation is trivial relative to among-stock variation in the traits used to discriminate the stocks.

We know of no explicit tests of this assumption, although some authors have evaluated differences among age classes within samples to draw inferences about temporal variation (e.g. Carscadden and Leggett 1975; Misra and Carscadden 1984). A difficulty with this method is that similarities or differences among age classes can arise for reasons other than temporal variation within stocks, particularly if a mixed stock is being sampled. Our objective is to test explicitly whether the assumption of temporal stability is tenable for traits used in stock identification.

Our purpose in this paper is to evaluate levels of temporal variation in meristic traits within a single, clearly defined stock. Meristic traits vary geographically in many fishes so they are widely useful in stock discrimination, and they are widely applied. Meristic traits are also useful because they tend to be ontogenetically stable, they are easily and inexpensively scored, and often they can be scored with little or no physical mutilation to high-value carcasses.

Atlantic salmon (*Salmo salar*) are a valuable and highly mobile resource that is subject to international and interprovincial exploitation (Lear and Sandeman 1980; Payne 1980; Reddin and Dempson 1986; Ruggles and Ritter 1980), and it provides a useful model species for our investigation. Individual stocks originate in rivers, they become mixed in offshore migrations, and they are exploited as mixed stocks in offshore fisheries. Optimal management requires that stock structure be integrated within an overall management strategy, and this has prompted numerous attempts to differentiate among populations using a variety of techniques such as electrophoresis (Child 1980; Payne et al. 1971; Payne 1980; Verspoor 1986), demographic characteristics (Power 1981; Reddin and Dempson 1986), scale characters (Lear and Sandeman 1980; Reddin and Misra 1985), morphometric and meristic traits (Claytor 1984; MacCrimmon and Claytor 1985, 1986), and combinations of these (Heggberget et al. 1986; Thorpe and Mitchell 1981). The meristic traits we examine in this study are those that are known to be the most variable geographically (Claytor 1984; MacCrimmon and Claytor 1985, 1986). They are, therefore, of greatest potential value in stock identification.

Materials and Methods

Specimens

To avoid confusing within-stock variability with among-stock variation, we conducted our study on the downstream migrants (smolts) of Western Arm Brook, Newfoundland, Canada. The smolts were collected at a counting fence located at the head of tide of the river. Western Arm Brook flows into St. Barbe Bay on the northwestern coast of Newfoundland (51°11′;21″N, 56°45′48″W) (Fig. 1). It is termed a "grilse" river because the smolts that leave the watershed remain at sea only 1 yr and return to fresh water as "grilse," or one sea-winter salmon, which are smaller than multiple sea-winter fish. Hence the spawners of each year are members of the same smolt migration, and only variation in the age to smoltification (between 3 and 6 yr in Western Arm Brook) maintains genetic continuity among spawning runs.

The smolts were obtained by systematic sampling within each downstream migration between 1977 and 1986 (e.g. every 200th fish in the downstream run is sacrificed and preserved), and fish were individually tagged when sampled. Hence, both between-year and within-year components of temporal variability can be addressed. The fish of 1978 were unavailable for analysis, so there is a gap in the data record, but the sequence from 1979 to 1986 is continuous. Length, weight, and sex were scored at the time of capture, and scale samples were removed for ageing. Age estimates allow us to evaluate temporal variation in terms of year of hatching (cohort) as well as year of migration (smolt run).

Traits

The meristic character set includes vertebrae, gill rakers, pectoral fin rays, dorsal fin pterygiophores, and anal fin pterygiophores. Vertebrae and dorsal and anal fin pterygiophores were counted from radiographs (Kodak Industrex M-2 film). Gill rakers and pectoral fin rays were counted directly from preserved specimens using a binocular dissection microscope.

All vertebrae were counted including the upturned or ural centra, but excluding the urostyle, following MacGregor and MacCrimmon (1977).

Dorsal and anal fin pterygiophores were enumerated from X rays because it was deemed more reliable to score these internal structures than the external fin rays. Pterygiophore number, both dorsal and anal, was scored as the number of distinct, narrow, extended pterygiophores. This excludes the anterior diffuse or short pterygiophore, in both the dorsal and anal fins. In salmon the anterior three rays are rudimentary and are associated with the first pterygiophore, which was not counted. Therefore, when comparing this method with studies where both primary and rudimentary rays were counted, 2 must be added to the number of pterygiophores. When comparing with studies where only primary fin rays were counted, the number of primary fin rays equals the number of pterygiophores.

Pectoral fin rays were scored on the left side except when the left was damaged. All rays were enumerated, including the small posteriormost ray, according to MacGregor and MacCrimmon (1977).

Gill rakers were scored as the total number including all rudiments on the first (anterior) left gill arch. The gill arch was removed and the rakers were counted according to MacGregor and MacCrimmon (1977). Rakers on the right arch were scored if the left was damaged or deformed.

Data Organization and Analysis

The data were organized in two ways for analysis (Table 1): by smolt run in which all smolts caught migrating downstream in the same year are grouped and by cohort in which fish are combined that hatch in the same year, but that smoltify 3, 4, 5, or 6 yr later. These data partitions give two ways of testing for temporal variability in the meristic traits, by cohort and by run.

Since meristic traits are discrete, analysis of frequency distributions was principally by contingency χ^2. Initial crosstabulations were calculated for all data pooled to evaluate which phenotypic values should be combined to avoid sparse cells in the χ^2 analyses. The overall distributions and the values pooled for each trait are shown in Table 2. Fish ranged in age from 3 to 6 yr, but 6-yr-old smolts are rare (3 of 1377 fish). To further

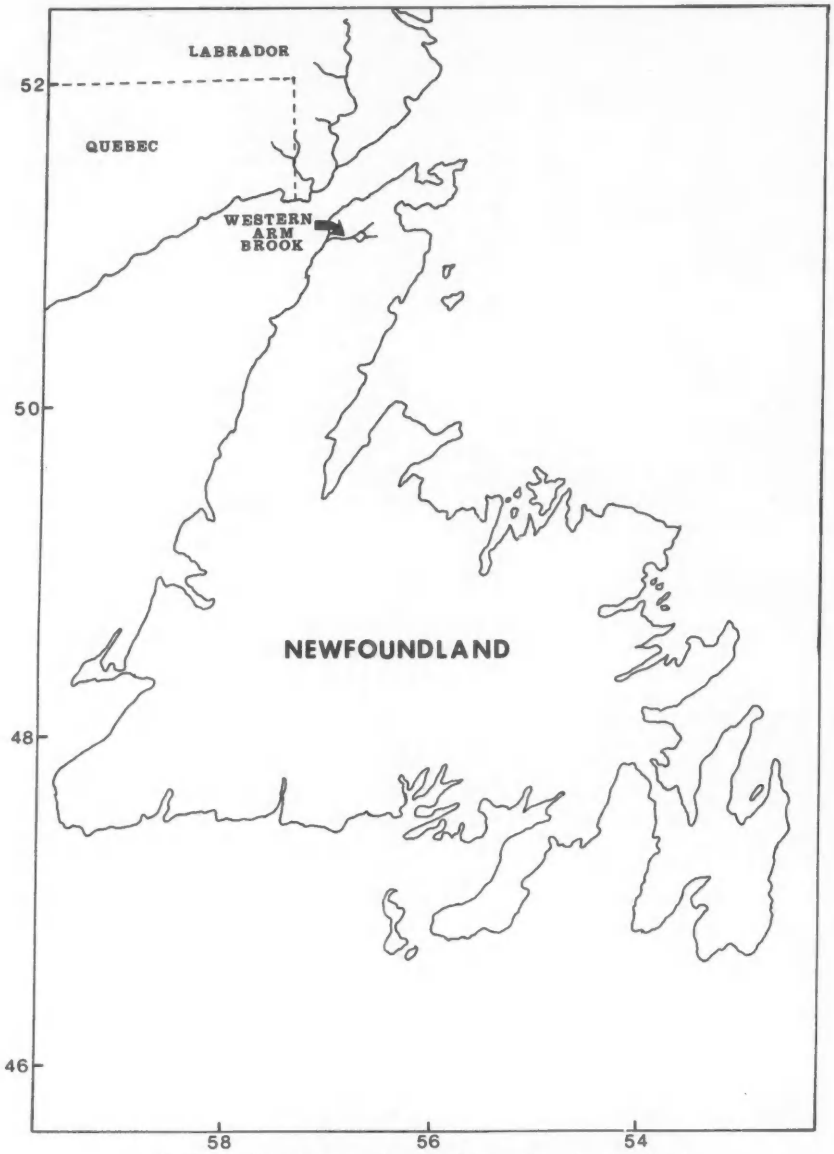

FIG. 1. Location of Western Arm Brook in northern Newfoundland.

avoid sparse cells the 6-yr-old smolts were therefore pooled with 5-yr-old smolts.

Sampling Reliability and Within-Group Variability

Before considering variation *among* the cohorts or runs, we evaluated sampling reliability, and variability with respect to sex, age, growth rate, and migration sequence, *within* the sampling units.

It is possible to evaluate sampling reliability with our data because fish of the same cohort were sampled several times. If our sampling is reliable, and assuming that no natural selection acts, we expect estimates of phenotype distributions to remain stable within a cohort from one year to the next. We tested this expectation by comparing phenotype distributions of fish of the same cohort but caught in different runs. Because there are fewer 3- and 5-yr-old smolts than those of age 4, sparse cells restricted us to 19 comparisons. Of these, there are four significant differences in phenotypes between samplings: one relating to vertebrae, one to anal pterygiophores, and two to dorsal pterygiophores. Given that 15 of 19 comparisons are nonsignificant, and that the variation among cohorts and runs is clearly much greater than this variation within cohorts (see below), we conclude that our sampling is adequate.

For males, sex was scored as immature, precocious, or maturity unknown. There were no differences among the three

TABLE 1. Sample sizes of smolts used in the study, partitioned by smolt run and by cohort.

	Sample size
Smolt run	
1977	142
1979	195
1980	230
1981	130
1982	145
1983	125
1984	120
1985	130
1986	160
Total	1377
Smolt cohort	
1973	33
1974	122
1975	140
1976	174
1977	221
1978	145
1979	89
1980	158
1981	117
1982	116
1983	54
Total	1369

TABLE 2. Phenotypic classes and frequency distribution of the meristic traits. Classes underlined are pooled in statistical analysis. Missing values are excluded. Traits: VERT = number of vertebrae; DORA = number of dorsal pterygiophores; ANRA = number of anal pterygiophores; PECRA = number of pectoral fin rays; GIRA = number of gill rakers on the anterior left arch.

VERT	Phenotype	56	57	58	59	60		
	N	31	229	714	360	34		
DORA	Phenotype	10	11	12	13	14		
	N	1	147	911	300	5		
ANRA	Phenotype	8	9	10	11	12		
	N	3	339	868	150	2		
PECRA	Phenotype	11	12	13	14	15		
	N	1	0	138	1096	141		
GIRA	Phenotype	16	17	18	19	20	21	22
	N	10	108	423	519	268	43	4

TABLE 3. Partitioning of the gill raker/age relationship to determine in which smolt run(s) gill rakers are not independent of age.

Smolt run	Chi-square	Probability
1977	$\chi^2_{(3)} = 2.32$	$P > 0.05$
1979	$\chi^2_{(3)} = 4.18$	$P > 0.05$
1980	$\chi^2_{(3)} = 13.73$	$P < 0.01$
1981	$\chi^2_{(3)} = 1.12$	$P > 0.05$
1982	$\chi^2_{(3)} = 3.20$	$P > 0.05$
1983	$\chi^2_{(3)} = 2.89$	$P > 0.05$
1984	$\chi^2_{(3)} = 0.66$	$P > 0.05$
1985	$\chi^2_{(3)} = 4.03$	$P > 0.05$
1986	$\chi^2_{(3)} = 2.05$	$P > 0.05$

TABLE 4. Tests for temporal variation in the meristic traits by smolt run and by cohort.
See Table 2 for definition of traits.

Trait	Chi-square	Probability
	Smolt run	
VERT	$\chi^2_{(16)} = 38.68$	$P < 0.001$
DORA	$\chi^2_{(16)} = 68.62$	$P < 0.001$
ANRA	$\chi^2_{(16)} = 47.20$	$P < 0.001$
PECRA	$\chi^2_{(16)} = 38.02$	$P < 0.005$
GIRA	$\chi^2_{(24)} = 43.61$	$P < 0.01$
	Cohort	
VERT	$\chi^2_{(20)} = 61.74$	$P < 0.001$
DORA	$\chi^2_{(20)} = 55.88$	$P < 0.001$
ANRA	$\chi^2_{(20)} = 56.36$	$P < 0.001$
PECRA	$\chi^2_{(20)} = 63.26$	$P < 0.001$
GIRA	$\chi^2_{(30)} = 49.99$	$P < 0.025$

classes with respect to the meristic variables, nor with respect to age. Therefore, all males were pooled in subsequent analyses.

Homogeneity between males and females was evaluated for each trait. There is no evidence of association between sex and any trait, so males and females were pooled in subsequent analyses. The sexes are homogeneous for age distribution as well.

Further analyses showed that all trait distributions are independent of age; however, the χ^2 value for gill rakers and age is at the borderline of statistical significance ($\chi^2_{(6)} = 11.83$; $0.05 < P < 0.10$). This relationship was further investigated by partitioning χ^2 according to smolt run (Table 3). Sample sizes within runs are smaller, and sparse cells became a problem, so the contingency tables were collapsed by pooling fish of age 5 with those of age 4. There is no evidence of association between the number of gill rakers and age except in the 1980 smolt run where 4- and 5-yr-old fish have more rakers than those of age 3. This suggests that for the 1980 smolt run, the number of gill rakers increases with age. However, the same pattern would be expected in the other runs if this were a general phenomenon, and given the number of comparisons made, this one significant association could be a Type 1 statistical error. There is no evidence of a consistent relationship between gill rakers and age, and there is no evidence of heterogeneity with age for any other trait, so we conclude that the traits are ontogenetically stable. Hence, we pool the age groups within runs for subsequent analyses.

To examine whether trait distributions are related to growth rates, comparisons of mean lengths and mean weights were made among the values of each meristic trait by analysis of variance. Comparisons were made only within groups of fish of a common age and common cohort to avoid confounding temporal effects with size (growth) effects. Sample sizes range from 20 to 132 among the 19 comparisons that were possible. There is no evidence of difference in either length or weight among the phenotypic classes for any of the traits.

The final question we asked in terms of heterogeneity *within* the sampling units was whether there is evidence of differences in the meristic attributes of smolts leaving Western Arm Brook at different times during the run. Since we know the sequence of capture of all specimens, it is possible to partition the fish of each run into three groups of equal size, corresponding to early, midrun, and late-migrating fish. Only 1 of the 45 contingency tables so constructed was significant, which is no

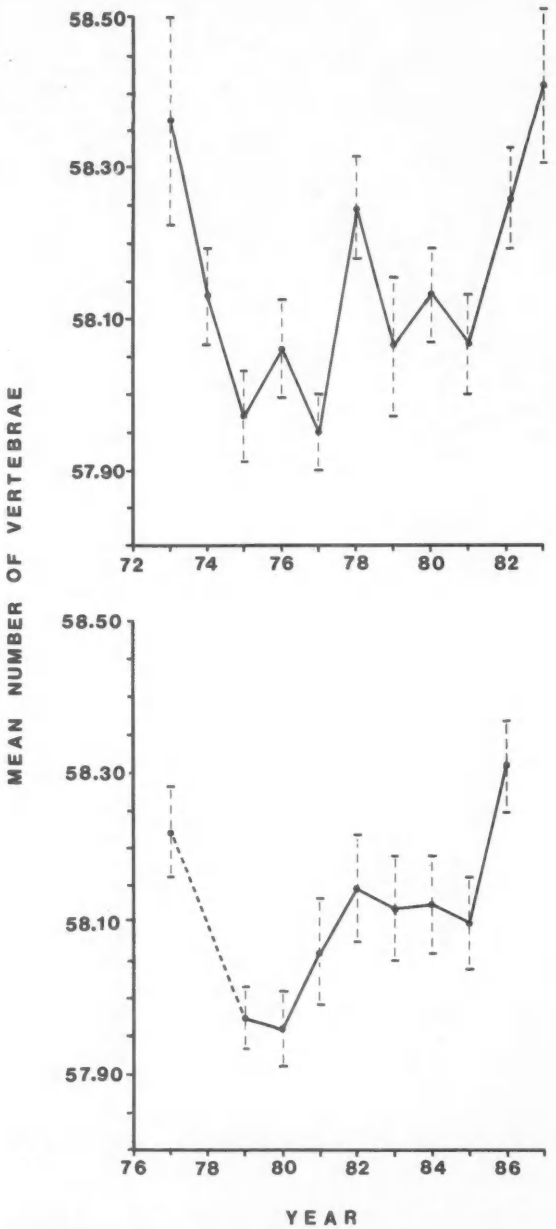

FIG. 2. Mean number of vertebrae plotted on cohort (upper panel) and smolt run (lower panel). Vertical bars represent one standard error above and below the mean. No information is available for the 1978 smolt run (broken line in lower panel).

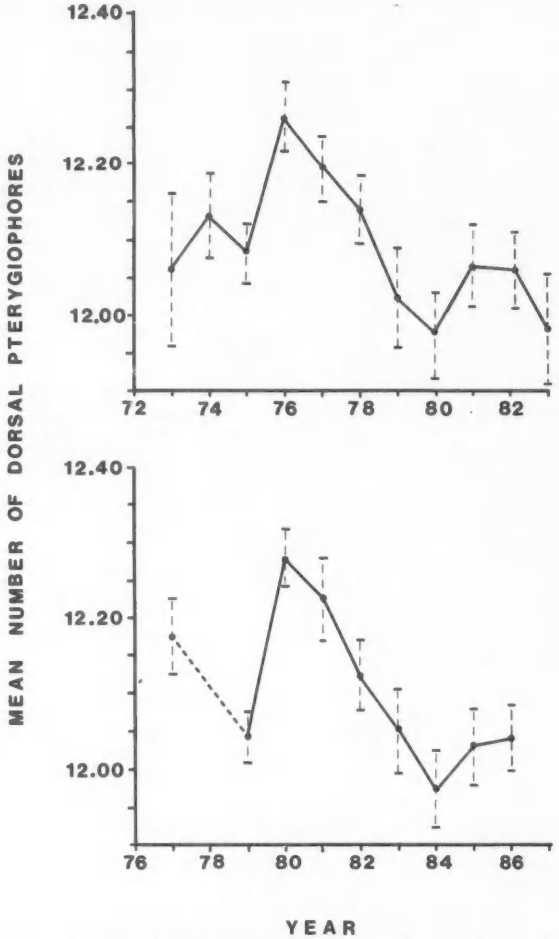

FIG. 3. Mean number of dorsal pterygiophores, plotted as in Fig. 2.

greater than could be due to chance. Thus there is no evidence of differential distributions of the traits among fish moving downstream at different times.

Results

To test the null hypothesis of temporal stability in the meristic traits, contingency χ^2 values were calculated for each meristic trait across smolt runs and cohorts separately. The null hypothesis is strongly rejected for each trait for both data partitions (Table 4). Hence there is highly significant variation over time in all traits within the Western Arm Brook stock.

To examine the nature of this temporal variation, the means of each trait were plotted over time by cohort and by run (Fig. 2–6). The plots show that the temporal variation is persistent across all years and that it is not due, for example, to any one particularly unusual year. This is true for each of the traits, and it is true whether the data are partitioned by cohort or by run. We conclude that temporal variation is the usual condition for these traits in the Western Arm Brook stock, with the obvious but important corollary that temporal stability is unusual.

If the traits are phenotypically correlated, then their independent examination (as was done above) will not allow us to detect underlying patterns of covariation and may lead us to conclude that there is more variability in the factors that determine phenotypes than actually exists. Hence we tested for independence among the traits, again using contingency χ^2. To increase power of the test through increased sample size, we used the total data set (i.e. pooled data from all years). The

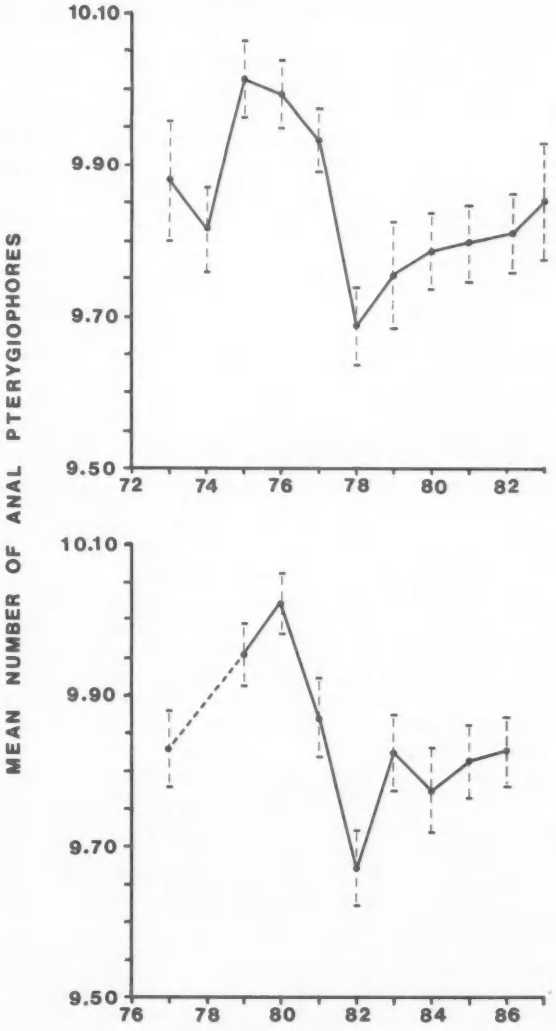

FIG. 4. Mean number of anal pterygiophores, plotted as in Fig. 2.

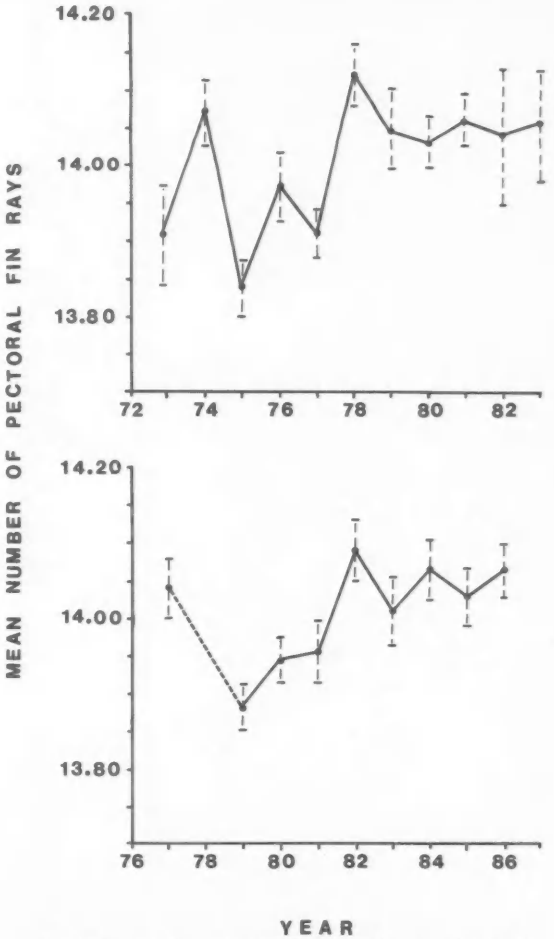

FIG. 5. Mean number of pectoral fin rays, plotted as in Fig. 2.

results (Table 5) show that 5 of the 10 test statistics are significant. Inspection of deviations from expectation for the significant contingency tables (not shown) indicates that there is a positive relationship between numbers of parts for most of the traits, which is consistent with the notion of common determination. An exception is the relationship between gill rakers and anal pterygiophores, in which no pattern is evident. Hence the evidence suggests that there is some common determination of the variation for some of the traits.

Finally, in order to evaluate whether the variation in phenotypes is related to environmental variation, correlation coefficients were calculated between means of the meristic traits by cohort and (i) measures of environmental variation over time and (ii) measures of biological variation in the Western Arm Brook stock. The environmental attributes include two measures of discharge (total and summer) and five measures of temperature (degree days > 4°C, degree days > 7°C, mean annual temperature, mean summer temperature, and mean winter temperature). The biological characteristics of the stock include mean age of the smolts arising from a cohort, the smolt density, mean smolt length and weight, mean condition factor, sex ratio, the proportion of young (3-yr-old) smolts arising from a cohort, and the proportion of eggs that survived to produce 3-yr-old parr. The data for all these variables are from Chadwick (1987).

Only 6 of 75 correlation coefficients are significant at the 0.05 probability level (Table 6), which is marginally more than could be due to chance alone, and none are highly significant. Four of the significant correlations involve pectoral rays (negatively correlated with two measures of summer temperature and with two measures of the mean size of smolts of the cohort). Gill raker number is negatively correlated with mean length of smolts of the cohort and with the proportion of young smolts (Table 6). These significant correlations are difficult to interpret biologically, but they do suggest that pectoral rays and gill rakers may be more subject to environmental effects than are the other traits. Nevertheless the predominant pattern is that the meristic traits vary independently of discharge and temperature and independently of variation in the biological characteristics of the smolt runs.

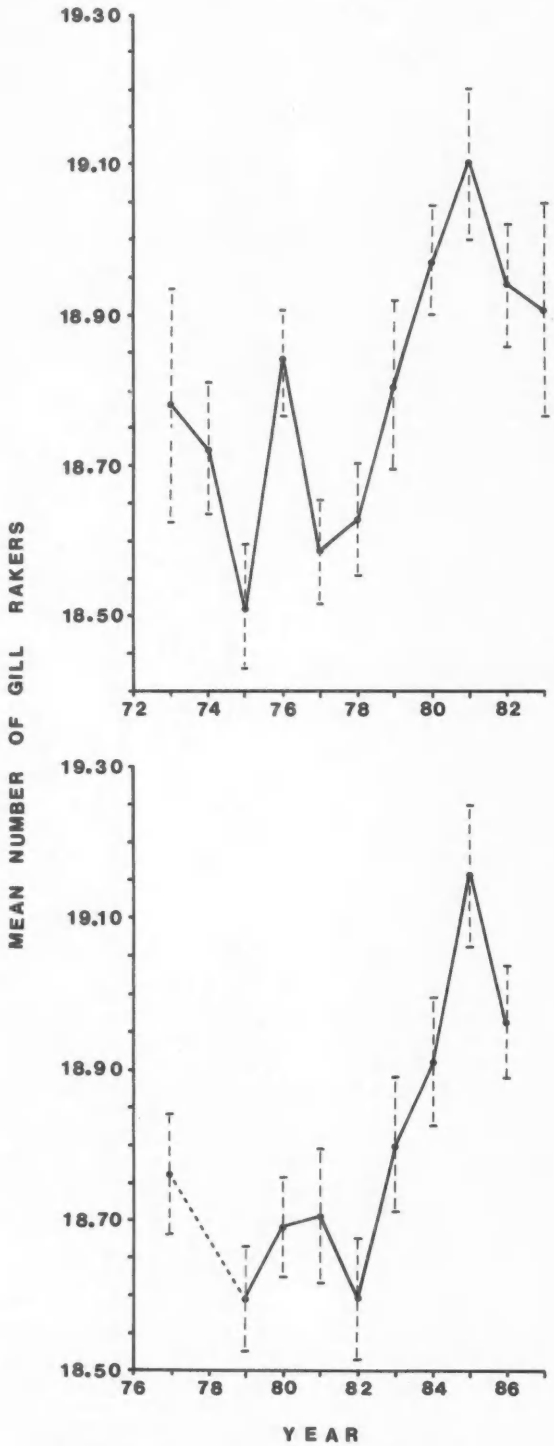

FIG. 6. Mean number of gill rakers, plotted as in Fig. 2.

Discussion

The five meristic traits that we examined show homogeneity with respect to sex, age, growth rate, and sequence of migration *within* the data partitions (smolt runs, cohorts). But the null hypothesis of temporal stability *among* these partitions is strongly rejected for every trait.

To place this temporal variation in context of geographic differences among stocks of Atlantic salmon, the ranges in means for both types of variation are shown in Fig. 7 (data for geographic variation were compiled from papers cited in the references, principally Claytor 1984). Relative to expectation, the variation over time at Western Arm Brook is very large — in fact, the observed means for dorsal pterygiophores extend beyond the previously documented range of means for this trait in North America (Fig. 7).

Another way to place the temporal variation in context of geographic variation is to ask whether it is possible to use the temporal variation to discriminate among smolt runs as though they were geographic stocks. Discriminant analyses on meristic traits of Atlantic salmon have resulted in correct classification percentages in the 20–80% range, depending on the traits used and on whether the geographic focus is fine (among rivers) or coarse (between continents) (Claytor 1984; MacCrimmon and Claytor 1985, 1986). Solely for comparative purposes then, we used standard software (SYSTAT, Wilkinson 1986) to compute discriminant functions among smolt runs on the basis of the five meristic traits. We attempted to discriminate among all nine smolt runs and achieved 21% correct classification. All five traits contributed significantly. However, the 21% correct classification rate represents only an 11% improvement over classification by chance alone, as measured by the kappa statistic of Titus et al. (1984). Hence the levels of temporal variation within Western Arm Brook are sufficiently large to allow statistically significant discrimination among runs, but only marginally better than could be due to chance alone, and at a lower order of accuracy than that achieved among river systems (see MacCrimmon and Claytor 1985).

We know of only two other studies wherein temporal variation in meristic phenotypes in teleost fishes has been evaluated over comparable time periods. Schmidt (1921) examined variation in the number of vertebrae, pectoral fin rays, and hard dorsal rays of the eelpout (*Zoarces viviparus*) over a 10-yr period at several sites in the Baltic Sea. His results are parallel to our findings for Atlantic salmon; all traits vary greatly over time, they tend to vary independently, the variation occurs among, not within, cohorts, and the variation over time within populations is large relative to geographic variation among populations. In addition, Schmidt (1921) found that the number of pectoral rays varies synchronously for two populations separated by about 50 km in adjacent fjords. He found similar synchrony for hard dorsal rays in a comparison between two other, more widely separated populations that have distinctly different (nonoverlapping) distributions for this trait. Schmidt (1921) concluded that pectoral rays and hard dorsal rays are influenced by broad-scale climatic events during development.

The results of Blouw and Hagen (1987) for temporal variation in dorsal spine number in two coexisting sticklebacks, the fourspine (*Apeltes quadracus*) and the ninespine (*Pungitius pungitius*), are in sharp contrast. Although there are statistically significant changes over a 10-yr period in both species, the changes are small relative to geographic variation, they take place at or near reproduction, they are not related in any obvious

TABLE 5. Tests of independence by contingency χ^2 of each meristic trait from all others. Table entries are χ^2 (df) (P). See Table 2 for definition of traits.

	VERT	DORA	ANRA	PECRA
DORA	6.48 (4) (>0.05)			
ANRA	8.17 (4) (>0.05)	35.11 (4) (<0.001)		
PECRA	19.18 (4) (<0.001)	10.09 (4) (<0.05)	0.96 (4) (>0.05)	
GIRA	10.40 (6) (>0.05)	4.67 (6) (>0.05)	16.26 (6) (<0.05)	16.22 (6) (<0.05)

TABLE 6. Correlation coefficients between the means of the meristic traits and scores of environmental variables and scores of biological variables of the Western Arm Brook stock. Correlation coefficients significant at $P = 0.05$ are indicated by asterisks. Deg Day 4 = number of degree days above 4°C; Deg Day 7 = number of degree days above 7°C; Mean Temp = annual mean daily temperature; Summ Temp = summer mean daily temperature; Wint Temp = winter mean daily temperature; Tot Dschg = total annual water discharge; Sum Dschg = summer water discharge; Density = density of smolts; Mn. Sm Age = mean age of smolts in the run; Mn Sm Len = mean length of smolts in the run; Mn Sm Wt = mean weight of smolts in the run; Condition = condition factor of smolts in the run; Sex Ratio = sex ratio of smolts in the run; % 3 Yr Sm = percentage of 3-yr-old smolts in the run; Egg Surv = percentage of egg survival to 3-yr-old parr. See Table 2 for definition of traits.

Variable	VERT	DORA	ANRA	PECRA	GIRA
Environmental					
Deg Day 4	−0.379	0.092	0.369	−0.550	−0.218
Deg Day 7	−0.271	0.159	0.452	−0.645*	−0.419
Mean Temp	−0.298	−0.365	−0.126	0.167	0.330
Summ Temp	−0.250	0.106	0.528	−0.703*	−0.329
Wint Temp	−0.309	−0.202	−0.408	0.436	0.366
Tot Dschg	−0.391	0.113	−0.041	0.024	−0.143
Sum Dschg	0.195	0.002	−0.164	−0.112	−0.484
Biological					
Mn Sm Age	0.356	−0.229	−0.329	0.508	0.629
Density	−0.374	0.014	−0.020	0.220	0.014
Mn Sm Len	0.041	0.370	0.594	−0.751*	−0.658*
Mn Sm Wt	0.035	0.317	0.608	−0.696*	−0.471
Condition	0.163	0.143	0.222	−0.290	−0.020
Sex Ratio	0.353	0.040	−0.526	0.065	−0.263
% 3 Yr Sm	−0.408	0.324	0.364	−0.545	−0.692*
Egg Surv	−0.208	0.558	0.172	−0.156	−0.448

way to variation in physical or biotic environmental factors, and there is no evidence of covariation between the species. Blouw and Hagen (1987) concluded that dorsal spine number for both species is strongly constrained by natural selection to local equilibrium values that are highly variable geographically, but relatively stable temporally.

There is evidence of demic structure and genetic differentiation of Atlantic salmon within the River Alta, Norway (Heggberget et al. 1986), and although the River Alta has a drainage basin almost four times that of Western Arm Brook, demic structure may exist in Western Arm Brook as well. If so, it is possible that temporally varying contributions from demes that are differentiated with respect to the meristic traits contribute to the variability among years. However, if this were the case, we expect more than one run of fish (of which there is no evidence) and differences in morphology between the early- and late-running smolts (also no evidence). Precocious males are homogeneous with their immature counterparts for the meristic traits, so it is unlikely that differential reproduction of precocious and grilse males contributes. Temporally varying natural selection for the meristic traits may contribute to the variation over time. If so, it must be strong, since the phenotypic distributions shift detectably each year.

To resolve which factors govern the changes in meristic phenotypes from year to year, and in what measure, it is clearly necessary that heritability estimates of the traits be made and that environmental influences be investigated by detailed sampling of both phenotypes and environments through development, or by controlled experiments.

Our results indicate that caution should be exercised if meristic traits are to be used to infer stock structure in Atlantic salmon fisheries. However, neglect of within-group variation is not unique to meristic traits, nor to Atlantic salmon — it is widespread in stock identification studies. We examine within-stock variation in morphometric traits in a subsequent paper wherein we reach the same general conclusion that morphological traits are temporally variable in the Western Arm Brook stock. Evidence is accumulating across a variety of organisms that at least some electrophoretic traits are subject to temporal variation, sometimes in association with environmental variation (e.g. Day et al. 1980; Hilbish and Koehn 1985; Jacobson et al. 1986; Johnson 1977; Koehn et al. 1971; Mork and Sundnes 1985; Philipp et al. 1985; Smith 1979). It is also well established that scale characteristics, which are used to identify continental origin of salmon in the Greenland international fishery, vary among years (Lear and Sandeman 1980). Hence the scale information that is to be used for stock identification in any year can only be gathered from tagged salmon taken in the Greenland fishery in that year. It is clearly undesirable to reestimate classification functions annually, but given the inherent variability in scale characteristics, there is no recourse. If our results are general, then repetitive sampling and reevaluation

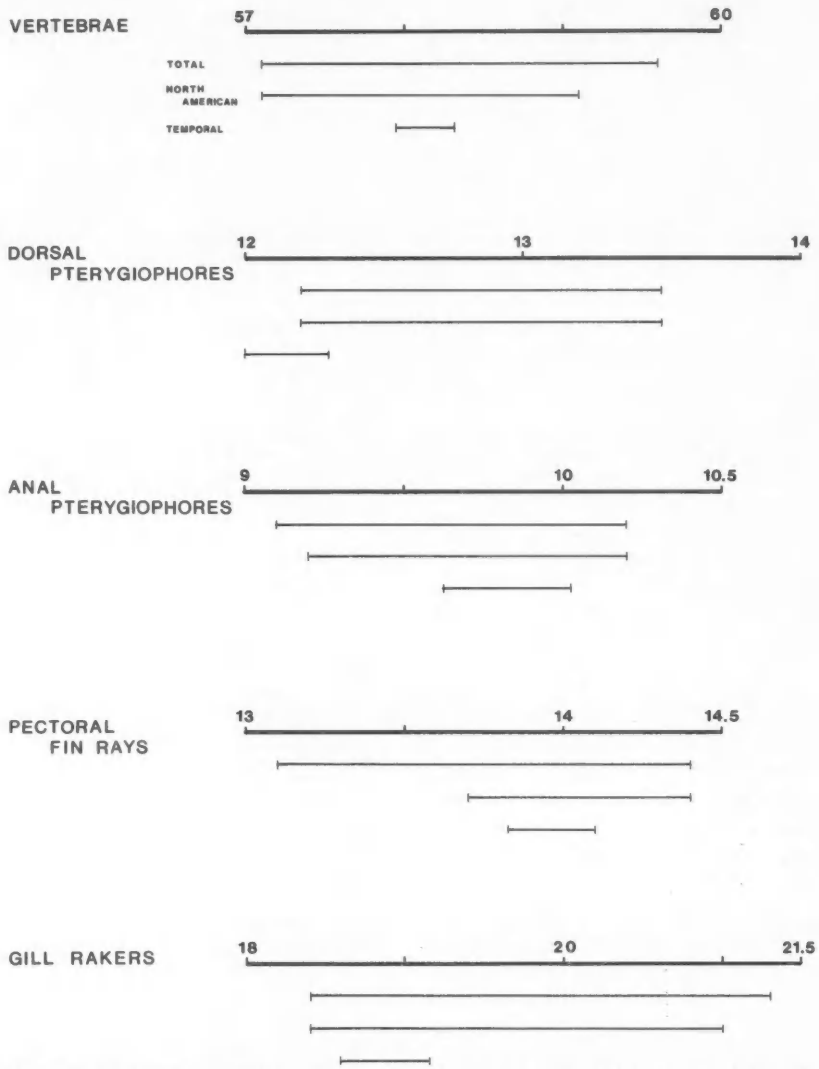

FIG. 7. Comparison of geographic and temporal variation in the five meristic traits. The first line for each trait gives scale, the second represents the range of means for the total North American and European distribution of Atlantic salmon, the third represents the range of means for North American populations only, and the fourth represents the range in means over time within the Western Arm Brook stock. Data were compiled from papers in the references.

of classification functions may be equally necessary for meristic and other traits.

We conclude that any stock identification attempts that do not address within-stock variability may be subject to error. The magnitude of the error will depend on the magnitude of temporal variation relative to among-stock variation, on the proportion of traits that show temporal variation, and on the degree to which the stocks in the fishery vary synchronously for the traits in question. More studies are needed to evaluate the generality of our findings and to discern the causes of the variation.

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Open Sea Releases of Atlantic Salmon Smolts, *Salmo salar*, in Central Norway, 1973–83

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Gunnerød, T. B., N. A. Hvidsten, and T. G. Heggberget. 1988. Open sea releases of Atlantic salmon smolts, *Salmo salar*, in central Norway, 1973–83. Can. J. Fish. Aquat. Sci. 45: 1340–1345.

Low returns of adult Atlantic salmon (*Salmo salar*) from releases of hatchery-reared smolts initiated experiments to increase recapture rates in the River Surna. A hypothesis that heavy postsmolt mortality due to predation could be reduced by guiding the smolts through coastal areas was tested. Three sites of release were selected: in the Surna, in the fjord, and on the coast off the Surna. Offshore releases gave 111% higher total recapture rates compared with river releases. The remote migration system did not seem to be disturbed by open sea releases, but the number of strayers to rivers other than the Surna increased significantly with the distance between point of release and the native river. Site of release of smolts was more important than genetic background of the fish for the site of recaptures of adult salmon.

Les faibles remontes de saumons de l'Atlantique (*Salmo salar*) adultes à la suite de lâchers de smolts élevés en pisciculture ont suscité des expériences axées sur une augmentation des taux de recapture dans la rivière Surna. L'hypothèse selon laquelle il serait possible de réduire la forte mortalité par prédation des post-smolts en guidant les smolts dans les zones côtières a été mise à l'essai. Trois sites de lâchers ont été choisis : dans la rivière Surna, dans le fjord et dans les eaux côtières devant la rivière. Les lâchers en mer ont donné des taux de recapture 111 % supérieurs aux lâchers en rivière. Le système de migration lointaine n'a pas paru perturbé par les lâchers en mer, mais le nombre de remontes dans des cours d'eau autres que la Surna a augmenté notablement avec la distance entre le point de lâcher et la rivière d'origine. En ce qui concerne les points de recapture des saumons adultes, le site de lâcher des smolts était plus important que le patrimoine génétique des poissons.

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Release of hatchery-reared Atlantic salmon (*Salmo salar*) smolts in Norwegian rivers has been used to compensate for lost habitats due to impoundment of streams since the late 1950's. About 288 000 hatchery-reared salmon smolts are stocked annually in Norway (Anonymous 1986), and the overall recapture of adult salmon from releases of Carlin-marked smolts is 2.2% (Rosseland 1979). Tags are reported by commercial and sport fisheries.

Because the recapture rate of hatchery-reared salmon is low, the cost of smolt production does not warrant current stocking programs (Hansen 1982). Recoveries of smolt-tags from birds and predatory fishes led to the hypothesis that different predators were responsible for significant mortality of postsmolts. Studies in the estuary of the River Surna and the River Orkla yielded predation rates of 24.8 and 20.0%, respectively, from cod (Hvidsten and Møkkelgjerd 1987; Hvidsten and Lund 1988).

In the Baltic Ocean, Carlin (1955) and Eriksson et al. (1981) reported a higher total recapture of adult salmon from releases into fjords than from river releases. To test the hypothesis that survival and migration pattern were affected by different sites of releases, groups of smolts approximately the same length were stocked at the same time in the Surna, the fjord, and in the sea each year between 1973 and 1983. A better survival of smolt released at sea was expected. However, based on earlier studies (Foerster 1936; Donaldson and Allen 1957; Quinn and Fresh 1984) on return of adult salmonids to site of smolt release, a lower rate of return to the Surna was expected from offshore releases than from the releases of smolts in the river.

Study Area

The River Surna, northwestern Norway, situated in Møre and Romsdal County, has a catchment area of 1190 km² (Fig. 1). The annual average discharge is 51.0 m³/s. Adult salmon returning to the Surna have normally spent 1–3 yr in the sea.

A total of 35 000 salmon smolts are annually released in the Surna to compensate for losses of natural smolt production resulting from hydroelectric production. Smolts are of the Surna strain, the parent fishes being caught in bag nets about 1 km outside the river mouth.

Materials and Methods

During 1973–83, tagged smolts were released in the river, the fjord, and in the sea (Fig. 2). The smolts were reared at Lundamo hatchery situated at the River Gaula about 100 km from the releasing site in Surna. They had not experienced "Surna water" before release. The smolts were anesthetized, measured for fork length, and tagged with Carlin tags attached by a double wire. After a least 1 wk of recovery the smolts were transported by truck to the release site at Surna, 20 km upstream

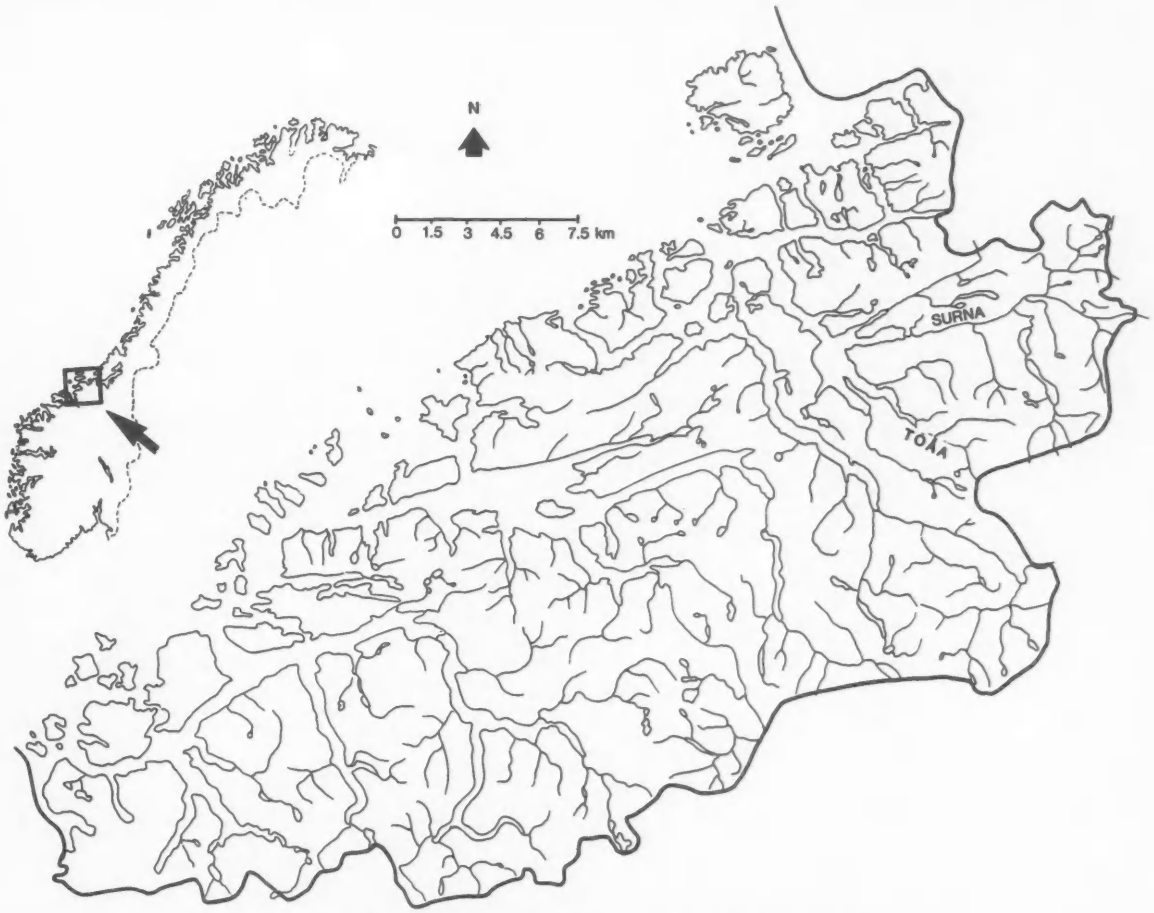

FIG. 1. River Surna and the River Toåa in Møre and Romsdal County.

from the mouth of the river. The date, numbers, mean lengths, and sites of released smolts are shown in Table 1. The smolt releases were performed within the period of the wild smolt run. The smolts were 2 yr old. Fjord releases were performed by trucking to piers. The sea-stocked smolts were transported by livefish boats 100 km northwest from the estuary. The smolts were transferred from a truck to a tank in the boat. Prior to the smolt transfer, the tank was filled with freshwater from the river mouth. The freshwater was gradually replaced by salt water as the smolts were transported to the sea. The sea releasing site was chosen off the coast where less pelagic predatory fishes such as the saithe (*Gadus virens*) and pollack (*G. pollachius*) at a depth of about 200 m are known to occur.

All recaptures refer to adult salmon of age 1–3 sea winters and are reported by commercial fishermen and anglers in marine and freshwaters.

In salt water, Atlantic salmon are caught by commercial fishing. Previous studies of stocks other than the Surna have shown that total exploitation rate for salmon in the sea may exceed 0.90 for 2-sea-winter salmon (Hansen 1986). In freshwater, Norwegian salmon are mainly exploited by sport fisheries. The rate of exploitation varies both between years and rivers. Hansen (1988) gave data from three Norwegian streams, indicating exploitation rates of 0.34–0.83. No analysis of exploitation rates of the Surna stocks exists, but based on other biologically similar stocks, exploitation rates of about 0.90 (sea) and 0.60 (river) are expected.

The chi-square method was used for statistical analyses of differences in numbers of recaptures of adult salmon released at different sites.

Results

The overall recaptures of adult salmon from total stockings of smolts were 1103 (3.0%) (Table 2). A total of 227 (1.9%) salmon were recaptured from the river releases, while 363 (3.1%) and 513 (4.0%) salmon were recaptured from the fjord and sea stockings, respectively. Overall sea and fjord stockings gave significantly ($\chi^2 = 94.9$ and $\chi^2 = 32.8$, $p < 0.001$) higher recapture rates than river releases, and the sea stockings also resulted in significantly ($\chi^2 = 16.5$, $p < 0.001$) higher recaptures of adult salmon than releases in the fjord. The highest rate of recapture followed stocking in the sea in 1976 which yielded 6.5%

The total number of adult salmon returning to the Surna from stockings of smolts in the river, fjord, and the sea during 1973–83 was not significantly different ($p > 0.05$).

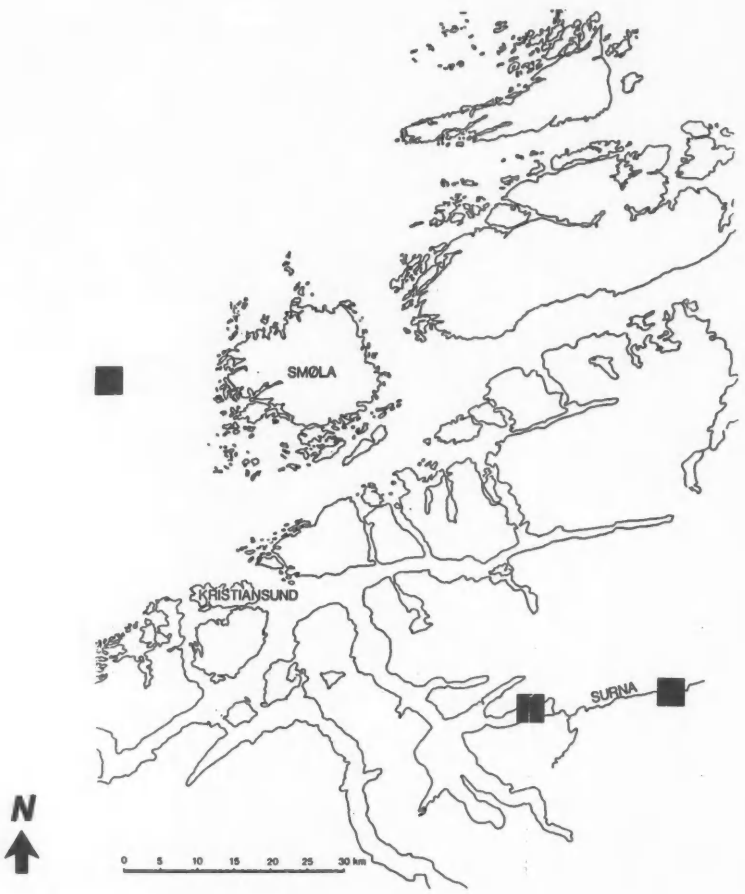

FIG. 2. Map showing the release sites (solid squares).

There were significantly higher recaptures of salmon from the fjord ($\chi^2 = 19.5, p < 0.001$) and sea ($\chi^2 = 10.4, p < 0.05$) stockings compared with river stockings in Møre and Romsdal County (Fig. 1). Recaptures from the fjord and sea stockings were not significantly different ($\chi^2 = 1.63, p < 0.05$).

The fjord and sea releases yielded significantly higher recaptures than river stockings also in other counties ($\chi^2 = 12.3$ and $\chi^2 = 88.9, p < 0.001$). No adult salmon from the river stockings were reported from rivers other than the Surna (Table 2). However, there were 6 (21.4%) and 13 (41.9%) strayers from the fjord and sea releases, respectively. In 1979 when the smolts were released in the neighbouring River Toåa, all river recaptures were reported from the Toåa (Fig. 1). The recapture rates of adult salmon from fjord- and sea-released smolts in rivers other than the Saurna were 6 and 13 specimens, respectively, compared with zero from the Surna releases in other rivers. This difference is significant ($\chi^2 = 6.1, p < 0.03$ and $\chi^2 = 12.2, p < 0.001$). In spite of differences in homing between the different release sites, the majority of recaptured salmon in freshwater are reported from the Surna. Hence, the return to the Surna from the fjord and sea releases is not incidental because salmon may ascend many other rivers in the area (Fig. 1). A random distribution of recaptures from the fjord- and sea-released smolts would have yielded more recaptures in rivers other than Surna along the coast of Møre and Romsdal.

Dicussion

Total recaptures of adult salmon from sea and fjord releases of hatchery-reared smolts were 111 and 63% better, respectively, than recaptures of river-released smolts. These results are in accordance with Carlin (1968), Larsson and Larsson (1979), and Eriksson et al. (1981). Eriksson et al. (1981) reported higher recaptures from fjord and sea releases of smolts than from river releases in the Baltic area.

TABLE 1. Date, site, number, and length of smolts released in the River Surna during 1973–83.

Year of release	Date (d/mo)	Site	No. released	Length (cm) Mean	Min.–max.
1973	11–26/4	Surna	3 000	15.6	13–29
	4/5	Sea	2 969	16.9	13–28
1976	16/5	Surna	999	16.8	14–23
	16/5	Fjord	995	17.4	14–23
	16/5	Sea	990	17.6	15–28
1977	27/5–7/6	Surna	1 978	17.1	14–21
	27/5–7/6	Fjord	3 933	17.0	13–29
1978	2/6	Surna	987	17.0	14–29
	1/6	Fjord	982	16.7	14–29
	1/6	Sea	1 982	16.7	13–27
1979	30/5	Toåa	2 000	17.5	14–30
	31/5	Fjord	2 000	18.1	14–32
	31/5	Sea	1 999	17.8	14–30
1980	30/5	Surna	914	17.1	14–32
	30/5	Fjord	931	16.9	14–30
	30/5	Sea	1 803	17.2	14–33
1982	24/5	Fjord	984	17.7	14–31
	24/5	Sea	1 970	18.7	14–32
1983	25/5	Surna	1 991	17.4	14–29
	25/5	Fjord	1 998	17.1	14–28
	25/5	Sea	993	16.6	13–25
Total		Surna*	11 869	16.9	—
		Fjord	11 813	17.3	—
		Sea	12 706	17.4	—

*Includes the releases in the river Toåa in 1979.

A minimum predation rate of 24.8% from cod was estimated in the estuary of Surna for one group of hatchery-reared smolts (Hvidsten and Møkkelgjerd 1987). In the estuary of Orkla the predation rate of wild and hatchery-reared smolts was estimated at a minimum of 20% from cod, and in addition, saithe was recognized as a smolt predator (Hvidsten and Lund 1988). Predation from birds on hatchery-reared smolts in the estuary of the River Eira was up to 10% (Reitan et al. 1987).

The increased survival of salmon released in the sea compared with river and fjord releases is likely to be associated with lower predation on the sea-released smolts. At ocean depths of 200 m at the releasing site in the sea, potentially predatory pelagic fishes such as the saithe and pollack are still found. One suggestion may be that higher recapture rates could be achieved by stocking the smolts outside the continental shelf, thereby avoiding predatory pelagic fishes.

The recapture rates are minimum values. One reason is that many fishermen do not report their catches of tagged salmon. Handling, anesthetizing, and the application of tags also cause additional mortality (Hansen 1981). Hansen (1981) reported 6 and 3% returns of unmarked and Carlin-tagged wild smolts, respectively, in the River Imsa. Isaksson and Bergman (1978) reported a 10% loss of Carlin-tags at Kallafjördur in Iceland. The total recaptures from wild unhandled 1-sea-winter salmon, including sea and river returns, probably exceed 20% for the Imsa strain (Jensen 1981).

The time of release is important to the survival of smolts and postsmolts (Hansen 1982; Hansen and Jonsson 1985; Larsson 1977). In central Norway, wild smolts descend the rivers in May and in some years the first week of June (Hesthagen and Garnås 1986; Garnås and Hvidsten 1985) which coincides with the spring runoff (Hesthagen and Garnås 1986). The releases in April 11–12, 1973, may not have coincided with the wild smolt run and may have in part caused the lowest recapture rates of all river releases in the Surna. A significant positive correlation between discharge at time of release of hatchery-reared smolts and recapture rate of adult salmon was found in the Surna and Gaula (Hvidsten and Hansen 1988).

The numbers of recaptures of adult salmon in the Surna from the releases in the river, fjord, and the sea were similar. The relatively high numbers of recaptures from sea-released smolts in the Surna contrast with the results of Eriksson et al. (1981) who observed significantly lower returns in the river from fjord and sea releases compared with river releases.

The present results indicate that the remote migration system was not disturbed by releasing smolts in the sea. The recapture data show that salmon released in the sea were recaptured locally both in salt water and in streams. Several studies of salmonids have shown that site of release of smolts in freshwater is important for the return of adults. In studies of several species of Pacific salmon, Shapovalov and Taft (1954), Donaldson and Allen (1957), and Quinn and Fresh (1984) found that the salmon not only returned to their native stream, but almost exclusively to the sites of release in the rivers. This study shows that the site of release in the sea is also important for the site of recapture, which is in accordance with the results from Sutterlin et al. (1982).

The proportionally lower homing to the river of sea-released smolts shows that the local migration pattern is affected by releases outside the natal river. Several theories are in existence to explain the mechanisms of migration in salmonids, but olfaction has been shown to be the most important cue for local navigation in salmonids (Hasler and Wisby 1951; Wisby and Halser 1954; Groves et al. 1968; Nordeng 1971; Toft 1975; Hasler et al. 1978; Westerberg 1982).

Two main theories exist: the imprinting of organic odors (Hasler and Wisby 1951; Hasler et al. 1978) and the pheromone hypothesis (Nordeng 1971), suggesting a genetically linked systen of pheromonal influence of conspecifics. Harden Jones (1968) presented a sequential hypothesis for home-stream detection, implying that the downstream migrants experience olfactory imprints that are stored sequentially. The sea-released smolts partially lost the sequential imprinting of olfactory stimuli when transported by boat. This may explain the reduced local river homing of sea-released smolts compared with river-released smolts. The proportion of sea-released smolts that apparently homed to the Surna may be explained by partial sequential imprinting and/or by pheromonal attraction to the natural salmon in the Surna. However, the 100% recaptures of smolts released in the Toåa show that possible pheromonal attraction of conspecifics in the Surna was insignificant for the navigation back to the Toåa of "Surna" salmon.

The higher recaptures of adult salmon from the stocking outside the river indicate that the imprinting of salmon smolts to the home river is not sufficient for the fjord- and sea-released smolts. There are no returns to other rivers from river-released smolts, and the rate of straying increases with the distance from the home river and the releasing site. This is in accordance with Carlin (1968) and Eriksson et al. (1981) and their descriptions of smolt releases in the Baltic Sea.

TABLE 2. Year, site, and numbers of released Carlin-tagged smolts of adult salmon.

Year of release	Site	No. released	Recaptures (no.) in Møre and Romsdal County				Recaptures (no.) in other counties			Total no. of recaptures	%
			River Surna	Other rivers	Sea	%	Rivers	Sea	%		
1973	Surna	3 000	1	0	9	0.3	0	29	1.0	39	1.3
	Sea	2 969	1	1	18	0.7	1	53	1.8	74	2.5
1976	Surna	999	3	0	13	1.6	0	7	0.7	23	2.3
	Fjord	995	0	0	12	1.2	0	17	1.7	29	2.9
	Sea	990	4	1	34	3.9	0	25	2.5	64	6.5
1977	Surna	1 978	3	0	29	1.6	0	34	1.7	66	3.3
	Fjord	3 933	15	3	67	2.2	3	88	2.3	176	4.5
1978	Surna	987	1	0	4	0.5	0	10	1.0	15	1.5
	Fjord	982	0	0	7	0.7	0	9	0.9	16	1.6
	Sea	1 982	6	1	24	1.6	2	66	3.4	99	5.0
1979	Toåa	2 000	5	0	12	0.9	0	18	0.9	35	1.8
	Fjord	2 000	5	0	32	1.9	0	27	1.4	64	3.2
	Sea	1 999	5	2	25	1.6	3	60	3.2	95	4.8
1980	Surna	914	1	0	7	0.9	0	11	1.2	19	2.1
	Fjord	931	2	0	14	1.7	0	10	1.1	26	2.8
	Sea	1 803	2	2	8	0.7	0	40	2.2	52	2.9
1982	Fjord	984	0	0	7	0.7	0	21	2.1	28	2.8
	Sea	1 970	0	0	30	1.5	0	67	3.4	97	4.9
1983	Surna	1 991	5	0	8	0.7	0	17	0.9	30	1.5
	Fjord	1 988	0	0	12	0.6	0	12	0.6	24	1.2
	Sea	993	0	0	5	0.5	0	27	2.7	32	3.2
Total	Surna[a]	11 869	19	0	82	0.7	0	126	1.1	227	1.9
	Fjord	11 813	22	3	151	1.3	3	184	1.6	363	3.1
	Sea	12 706	18	7	144	1.1	6	338	2.7	513	4.0

[a]Includes the releases in the River Toåa in 1979.

Results from the releasing program in the Surna between 1973–1983 show that the total recapture from sea stockings is 111% better than for river releases. The number of salmon returning to the river from the sea releases is also unchanged. The increase in recapture is due to greater catches in marine waters. It must be emphasized that high numbers of strayers to other rivers may represent a genetical problem, as the native salmon stocks might be disturbed.

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Preseason Forecasts of Sockeye Salmon (*Oncorhynchus nerka*): Comparison of Methods and Economic Considerations

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Bocking, R. C., and R. M. Peterman. 1988. Preseason forecasts of sockeye salmon (*Oncorhynchus nerka*): comparison of methods and economic considerations. Can. J. Fish. Aquat. Sci. 45: 1346–1354.

We compared the ability of two methods to forecast observed abundances of sockeye salmon (*Oncorhynchus nerka*) in Bristol Bay, Alaska, stocks from 1956 through 1980. We determined that the commonly used linear forecasting method, based on untransformed abundances of sibling age classes, overestimated sockeye abundances more frequently than did a nonlinear method. Furthermore, the nonlinear method produced mean forecasts that were closer to actual abundances of returns than forecasts from the linear method. We therefore recommend use of the nonlinear method. We also quantified significant economic implications of incorrect preseason forecasts for Bristol Bay sockeye. Deviations of actual escapements from the target are correlated with errors in the preseason forecast. Thus, large errors in preseason forecasts can influence potential long-term harvests and resulting economic benefits. In addition, annual changes in prices of set and drift gill net permits for fishermen were correlated with forecasted annual change in the abundance of sockeye recruits. This result is consistent with the hypothesis that preseason forecasts of sockeye salmon returns affect expectations by fishermen of economic rents (related to profits). Overestimates of abundance of recruits by current methods may thus subsequently lead to overinvestment by fishermen seeking to increase their share of expected rents.

Les auteurs ont comparé la capacité de deux méthodes à prévoir les niveaux d'abondance observés du saumon nerka (*Oncorhynchus nerka*) dans la baie Bristol (Alaska) de 1956 à 1980. Ils ont déterminé que la méthode de prévision linéaire la plus couramment utilisée, qui repose sur l'abondance non transformée des classes d'âge jumelles, surestimait plus fréquemment l'abondance des saumons nerkas qu'une méthode non linéaire. De plus, cette dernière méthode permettait d'obtenir des prévisions moyennes se rapprochant plus des niveaux d'abondance réels des remontées que la méthode linéaire. Les auteurs recommandent donc l'utilisation de la méthode non linéaire. Ils ont aussi quantifié les incidences économiques appréciables résultant de prévisions de pré-saison incorrectes des remontées du saumon nerka de la baie Bristol. Les écarts entre les remontées réelles et les valeurs cibles présentent une corrélation avec les erreurs des prévisions de pré-saison. D'importantes erreurs de ces prévisions peuvent donc influer sur les récoltes à long terme et les avantages économiques qui en résultent. De plus, les variations annuelles du coût pour les pêcheurs des permis de pêche aux filets fixes et dérivants présentaient une corrélation avec la variation annuelle prévue de l'abondance du recrutement du saumon nerka. Ce résultat est cohérent avec l'hypothèse voulant que les prévisions de pré-saison de la remontée du saumon nerka influent sur les attentes de rentes économiques des pêcheurs (donc sur leurs profits). La surestimation de l'abondance du recrutement des méthodes actuelles peut donc se traduire par un surinvestissement de la part des pêcheurs qui tendent à accroître leur part des rentes économiques prévues.

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The abundance of mature recruits in most sockeye salmon (*Oncorhynchus nerka*) stocks varies considerably from year to year (Rogers 1984). As a result, management agencies, fishermen, and fish processors have long recognized the desirability of making good forecasts of recruits (also called returns), prior to the onset of fishing each year. In this paper, we compare the performance of two methods for making these preseason forecasts for sockeye salmon in Bristol Bay, Alaska, and evaluate the economic implications of incorrect forecasts.

Numerous models have been developed to forecast fish abundance. The most commonly used methods are based on stock–recruit relationships or time-series analysis (e.g. Henderson et al. 1987; Stocker and Hilborn 1981; Mendelssohn 1980). Since 1960, the Alaska Department of Fish and Game (ADF&G) has issued annual forecasts of the following season's sockeye salmon returns to Bristol Bay. The standard ADF&G preseason forecasting method (Eggers et al. 1983) attempts to predict salmon abundances by river system and major age class (in Bristol Bay, sockeye salmon recruit mainly as 4-, 5-, or 6-yr-olds). Three component techniques are included in the standard ADF&G method: (1) spawner–recruit relationships, (2) sibling age class returns, and (3) smolt production and marine survival estimates (Eggers et al. 1983). Forecasts resulting from each of these components are weighted equally and averaged for each river system and age class.

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We focus here on the second component method, which deals with sibling groups, i.e. fish from the same brood class that mature in successive years. This method assumes, for example, that an abundant brood class will likely be reflected by a large abundance of 4-yr-old returns in year t as well as a relatively large abundance of 5-yr-olds in year $t + 1$. We concentrated on this sibling age-class component because Peterman's (1982) preliminary analysis of it showed that there were problems inherent in the method then being used and a modified method was derived. Details of the two sibling group methods (one linear and the other nonlinear) are given below in Part 1.

Good preseason forecasts of salmon returns help managers to achieve target escapement levels (desired number of spawners) which, if met, result in maximized long-term yields (Minard and Meacham 1987). Preseason forecasts most heavily influence managers' preliminary decisions on how many days of fishing will be allowed early in a fishing season; later in the season, the preseason forecast is given less weight (Yuen 1980; Minard and Meacham 1987). However, due to inherent constraints with in-season management, incorrect forecasts cannot be completely corrected for as the season progresses and such forecasts can thus lead to either overescapements or underescapements. Such constraints are particularly severe in sockeye stocks in Bristol Bay because 65% of the fish move through the commercial fishing areas in about 2 wk (Minard and Meacham 1987). This short season means that managers of these stocks have limited ability to adjust fishing plans to compensate for incorrect forecasts. One of our purposes here was to determine whether the ability to meet target escapements is related to incorrect preseason forecasts.

Accurate forecasts of the total run size also allow the harvesting and processing sectors of the salmon industry to make appropriate planning decisions regarding investment in supplies and manpower each year (Mathews 1971; Fried and Yuen 1987). Good forecasts help the industry to be neither underprepared for an unexpectedly large catch nor overprepared for an unusually small catch. Proper preparation is important because inadequate supplies or manpower can result in foregone fish, while excess inventory of supplies can increase operating costs and reduce profits (Crutchfield and Pontecorvo 1969; Tobolski 1983).

Our main purpose was to compare the performance of the two sibling age-class methods of forecasting. We did a retrospective analysis with Bristol Bay sockeye data to compare how well the linear and nonlinear methods would have performed historically. We then asked whether incorrect forecasts of abundance of sockeye salmon in Bristol Bay have important economic ramifications. In other words, is any difference in forecasts between the two methods likely to lead to important differences in economic benefits? To answer this, we evaluated the implications of incorrect forecasts for escapements and future yields and for investment behaviour of fishermen in the harvesting sector of the sockeye fishing industry.

Part 1: Empirical Analysis of Forecasting Techniques

We compared the ability of two different methods to forecast the abundances of sockeye that were actually observed in Bristol Bay sockeye stocks from return years 1956–80. These years are different from those analyzed in the following section on economics because *age-specific* data were only available for these years. As noted in the introduction, we focused on two versions of the sibling age-class method, described below.

Prior to 1986, ADF&G used linear fits of untransformed data on abundances of salmon returns to forecast the abundance of returns of age $n + 1$ in year $t + 1$ from the known abundance of returns of age n in year t (henceforth called the linear model) (Eggers et al. 1983). The structure of this model was

$$(1) \quad N_{t+1} = c + d \cdot N_t$$

where N_{t+1} is the abundance of $n + 1$-yr-old recruits in year $t + 1$, N_t is the abundance of n-yr-old recruits in year t, and c and d are parameters of the regression line. This procedure was repeated for each age group to generate a total forecasted annual return for each stock. This model using untransformed abundances is still used by fisheries managers in British Columbia (Sprout and Kadowaki 1987).

However, Peterman (1982) suggested that this method of relating these two sibling age classes could be improved simply by using logarithms of abundance instead of untransformed data. This is because empirical tests of the two methods showed that the logarithmic method best met the standard linear regression assumptions of (1) normally distributed Y-variates at each X value and (2) homoscedasticity of the data (Peterman 1982). This logarithmic model (henceforth called the nonlinear model) was

$$(2) \quad N_{t+1} = a \cdot N_t^b$$

where N_{t+1} and N_t are as in model (1) and a and b are parameters with b representing the degree of curvilinearity. By taking logs:

$$(3) \quad \log_e N_{t+1} = \log_e a + b \cdot \log_e N_t.$$

This nonlinear (or log-linear) model was derived from known sources of natural variability in survival and maturation processes, as detailed in Peterman (1982).

However, one cannot conclude that the model that fits the current data best is necessarily the preferred model for successful forecasting (Stocker and Hilborn 1981). Short historical data sets with large variance may result in a best-fit model being seriously biased. A more appropriate way to decide on the best forecasting method is to use the historical data to compare how well each method would have performed had it been used each year in the past. In other words, how frequently does each method overestimate or underestimate the actual returns, and by how much? This analysis of bias or systematic error, as well as precision, would differ from evaluations of forecasting methods that focused mainly on their precision (Stocker and Hilborn 1981).

Based on previous empirical work (Peterman 1981, 1982), we expected that the nonlinear forecasting method would perform better and that the linear method would tend to overestimate actual returns. This is because the linear method is influenced by occasional high abundance values that occur in the documented lognormal distribution of recruits, but the nonlinear method is not. We specifically tested two hypotheses concerning the relative forecasting powers of the linear and nonlinear methods. The first hypothesis was that the ADF&G method of forecasting using untransformed abundances of sibling age classes has led to more frequent overestimates during the given years than would have been the case had a nonlinear relation been used. The second hypothesis was that the magnitude of these overestimates would have been significantly smaller using a nonlinear forecasting method. We emphasized

overestimates because of their implications concerning economic investments, which are discussed in the next section.

Most past evaluations of forecasting methods have relied on using normal least squares procedures to evaluate which type of model best fits the data (Fox 1975; Uhler 1980; Schnute 1977). The methods we used are similar to the methods used by Stocker and Hilborn (1981) to compare how well different methods of forecasting catches of marine fish stocks would perform. The differences between their method and ours arise in the indicators used to measure forecasting performance. While Stocker and Hilborn (1981) computed a "*D*" statistic related to unexplained variation, we simply calculated average frequency and average magnitude of overestimates and underestimates. These indicators were considered to be of practical importance to fisheries managers and fishermen.

Methods

Data sources

Total returns or recruits (catch and escapements) for 10 Bristol Bay sockeye salmon stocks, by age and stock, were from summary data sheets provided by K. Parker and C. Meacham (ADF&G, Anchorage, Alaska), which were based on a series of ADF&G Technical Data Reports published for each year's returns. The stocks used in this analysis were the Kvichak, Branch, Naknek, Egegik, Ugashik, Wood, Igushik, Nuyakuk, Snake, and Togiak River sockeye (Fig. 1). Full descriptions of data for adult Bristol Bay sockeye, including allocation of catches among stocks, are found in Peterman (1982). The data for the stocks used in this analysis covered through return year 1980. A particular stock or age class was excluded from the analysis if (1) there were less than 20 yr of non-zero data on abundance for a particular age class or (2) the age class contributed less than 1% of the total return on average.

Analysis

For each of 10 sockeye salmon river systems in Bristol Bay, there exists a time series of actual observed N_t and N_{t+1} recruits for 24 yr, beginning in the 1956 return year. We independently forecasted abundances of sockeye salmon returns to Bristol Bay over this period using linear regressions of (1) untransformed abundances of recruits (equation 1) and (2) logarithms of abundances of recruits (equation 3).

For each of the 10 river systems, we used the following procedure. We first used the linear forecasting method and fit equation 1 to the first 10 yr of observed abundances by regressing N_{t+1} on N_t. The resulting equation was used to forecast the N_{t+1} abundance in year 11 from N_t in year 10. Next, this forecasted abundance of N_{t+1} recruits in year 11 was compared with the N_{t+1} actually observed. The time frame was then advanced 1 yr, the regression line was refit using the data up to and including the year 11, and a forecast was made of the abundance of N_{t+1} recruits for year 12. This procedure of advancing 1 yr, refitting the regression line, and forecasting the next year's abundance of recruits was repeated through the entire time series of data for each stock. A total of 10 Bristol Bay stocks were used in the analysis, with each stock having two distinct sibling age-class relations of 4-yr-olds to 5-yr-olds and 5-yr-olds to 6-yr-olds. Thus, a total of 14 annual estimates of N_{t+1} abundance were made for each of the 20 stock-specific age-class relations (10 stocks × 2 age groupings). This entire procedure was repeated for fitting and forecasting with the nonlinear method (equation 3).

We then calculated several indicators. Average frequency of overestimation was calculated as the percentage of the 20 cases in a given year in which overestimates occurred and then averaged over the 14 yr for which predictions were made. This gave a mean annual frequency of overestimation for all Bristol Bay sockeye stocks and age classes tested. The second indicator of

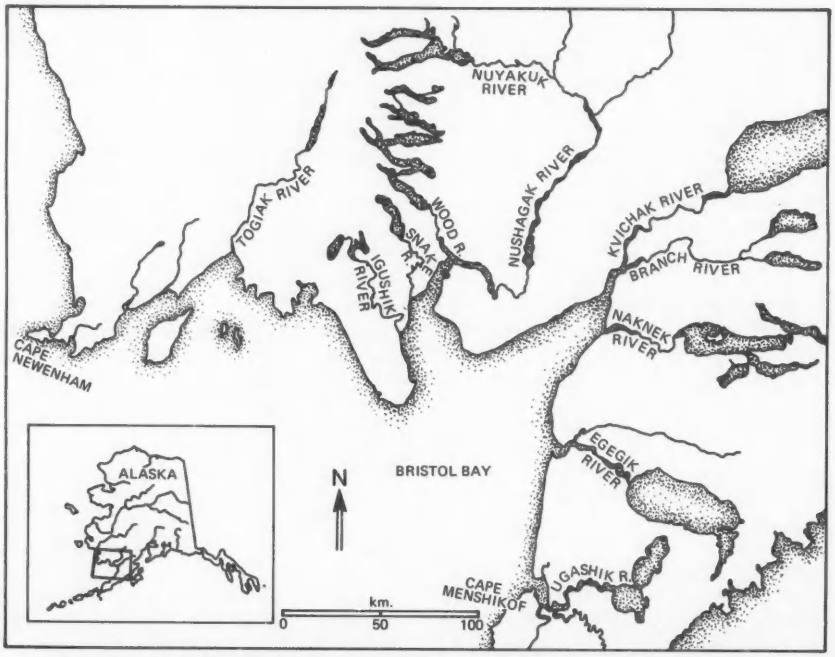

FIG. 1. Map of Bristol Bay, Alaska, showing location of sockeye salmon rivers included here.

TABLE 1. Comparison of performance of the linear and nonlinear methods of preseason forecasting of sockeye salmon returns to Bristol Bay, Alaska. Performance indicators show how each method would have performed had it been used for the two major age-class relations in 10 stocks over 14 yr. The frequency of overestimates was the mean percentage of cases in a given year in which preseason forecasts were above actual returns, averaged over the 14 yr in which predictions were made. *E/O* is the mean ratio of estimated (forecasted) recruit abundances (*E*) to observed recruit abundances (*O*), averaged over stocks and age-classes within a year, and then averaged over the 14 yr in which predictions were made. *E/O* was calculated for all years combined and then separately for years of overestimates and underestimates (bottom two rows). For means, probabilities (*P*) are given that the data are from the null model (i.e. 50% for the first row, 1.0 for other rows). For the difference between means, probabilities are given that the two means are the same ($n = 14$ for all cases).

Performance indicator	Means				*P* for difference between means
	Linear	*P*	Nonlinear	*P*	
Frequency of overestimates (% of cases)	58.2	0.091	38.6	<0.005	<0.001
E/O for all years combined	2.45	<0.001	1.14	0.14	<0.001
E/O for overestimates	3.74	<0.001	2.19	<0.001	<0.003
E/O for underestimates	0.62	<0.001	0.44	<0.001	<0.001

performance was average magnitude of error in forecasting. It was calculated as the mean ratio of the estimated N_{t+1} recruit abundance (E) over the observed N_{t+1} recruit abundance (O), averaged over the 20 estimates of abundance of N_{t+1} recruits for all stocks and age-class relations, and then averaged over the 14 yr. Average magnitude of error (E/O) was also calculated separately for years of overestimates or underestimates.

Paired difference *t*-tests were used to determine whether there was a significant difference between forecasting methods in measures of each performance indicator averaged over the 14 yr tested. The level of significance used throughout this study was $\alpha = 0.05$.

Results and Discussion

The linear method of forecasting overestimated significantly more frequently than the nonlinear method (Table 1). While the total mean frequency of overestimates of 58% by the linear method was not significantly different from 50%, the average forecast was significantly (2.45 times) above the actual recruitment (line 2, Table 1) whereas the mean forecast by the nonlinear method was not significantly different from actual recruitment (line 2, Table 1). To indicate stock abundance and hence magnitudes of errors, we calculated the mean observed abundance of recuits across stocks and years as 286 250 fish. However, for any given year and in any given stock, abundance of recruits and errors could be much smaller or larger.

As described in the introduction, this sibling age-class technique is only one part of the complex method used by ADF&G to forecast sockeye returns to Bristol Bay (Eggers et al. 1983). However, because the sibling age-class component's forecast is equally weighted along with the other components, ADF&G's complex method should have tended to overestimate returns. This was indeed the case during the period 1962–74 when the standard ADF&G forecast overestimated total sockeye abundances in ten of thirteen years (77%) (Eggers et al. 1983). From 1975 to 1982, however, the standard ADF&G forecast overestimated in just one of eight years (12.5%). This recent pattern of underestimates suggests that one or both of the other component techniques used in the standard ADF&G forecast (spawner–recruit relationships, smolt production and marine survival estimates) have underestimated total sockeye returns to Bristol Bay during the period 1975–82, thereby masking the tendency for the sibling age-class technique (linear method) to overestimate. The tendency to underestimate returns in recent years was possibly due to the dramatic increase in sockeye production since the late 1970's. Rogers (1984) and Eggers et al. (1984) discussed possible mechanisms for this observed increase in production; these include reduction of the Japanese high seas fishery and increased marine survival rates. Nonetheless, the use of the linear sibling age-class technique will tend to bias the preseason forecast higher than it should be.

When the mean magnitude of errors for each method was calculated separately for over- and underestimates, underestimates by the linear method tended to be closer to actual recruitment than underestimates by the nonlinear method (Table 1). But when the nonlinear method did overestimate recruits, forecasts were significantly closer to actual returns than the linear method (Table 1).

In summary, the results indicate that overestimations occurred more frequently using the linear method than would have been the case had the nonlinear method been used. Not only would the nonlinear method have overestimated less frequently, but its average forecasts would have tended to be significantly closer to the actual recruitment than forecasts by the linear method.

These results are specific to Bristol Bay sockeye and depend on the variability around the stock-specific age-class relations and the degree of nonlinearity of each true age-class relation. The robustness of our conclusions to these sources of change was evaluated in a separate study using Monte Carlo simulation (Bocking 1987). That study showed that the nonlinear method performed better than the linear method under a wide range of variances around the age-class relation and varying degrees of nonlinearity.

In the next section, we discuss some of the economic ramifications of errors in preseason forecasts of sockeye salmon returns to Bristol Bay.

Part 2: Economic Considerations of Incorrect Forecasts

With the notable exception of Mathews (1971), there have been few attempts to evaluate the economic benefits of preseason forecasts. Mathews estimated the benefits and costs of having more precise preseason forecasts, but did not examine the potential systematic errors or biases from either a biological or economic perspective. Here we address the effects that the frequency of over- or underpredictions might have on escapements and yields, and on investment by the harvesting sector.

We focused on the Bristol Bay commercial sockeye fishery, upon which the economy of the area is very dependent. The 1960–82 average ex-vessel value of the sockeye salmon fishery to fishermen was U.S. $39.5 million in constant 1982 dollars (Middleton 1983). The next most important salmon species in Bristol Bay, chum salmon (*O. keta*), was worth U.S. $1.9 million in constant 1982 dollars, followed closely by chinook salmon (*O. tshawytscha*) valued at U.S. $1.85 million (Middleton 1983). In numbers of fish, the Bristol Bay sockeye catch accounts for, on average, 63% of the Alaskan sockeye catch, 59% of the entire American sockeye catch, and 42% of the combined Canadian, American, and Japanese sockeye catch (1972–82) (International North Pacific Fisheries Commission 1978–85).

The inshore salmon catch is taken entirely by gill nets with drift gill net gear accounting for 90% of the entire Bristol Bay salmon catch, on average, and set nets accounting for the remaining 10% (Middleton 1983). In 1974, limits on the number of fishing gear permits were imposed on the Bristol Bay fishery. Since then, salmon permits have been freely transferable, but are assigned only to persons, not boats or corporations, and may not be leased.

It is exceedingly important that the Bristol Bay salmon processing industry has the appropriate manpower and equipment on hand before the season because (1) the season is very short, (2) the fish cannot be held more than a few days before processing, and (3) the area is far from sources of labour and equipment. These characteristics of the Bristol Bay sockeye fishery make accurate and timely fisheries management necessary to control the harvest and achieve escapement goals. However, like salmon management systems everywhere, escapement goals are seldom met precisely, as we will document below.

Management begins with a preseason forecast, which attempts to estimate abundance of the annual run and its timing for each individual stock returning to Bristol Bay (Fried and Yuen 1986). This is then followed by intense in-season management designed to correct quickly for errors in the preseason forecasts in order to achieve the desired target escapement.

Implications for Escapements and Yields

Methods

The annual escapement is the most important element in managing the sockeye salmon fishery in Bristol Bay. The target or optimum escapement is intended to yield the maximum number of harvestable recruits above replacement. If we assume that the managers' target escapement is indeed set at the optimum level, then escapements above or below this goal will produce suboptimal yields in the long run. Target escapements for Bristol Bay sockeye have varied among stocks and in some cases among years, depending on current parameter estimates (Middleton 1983).

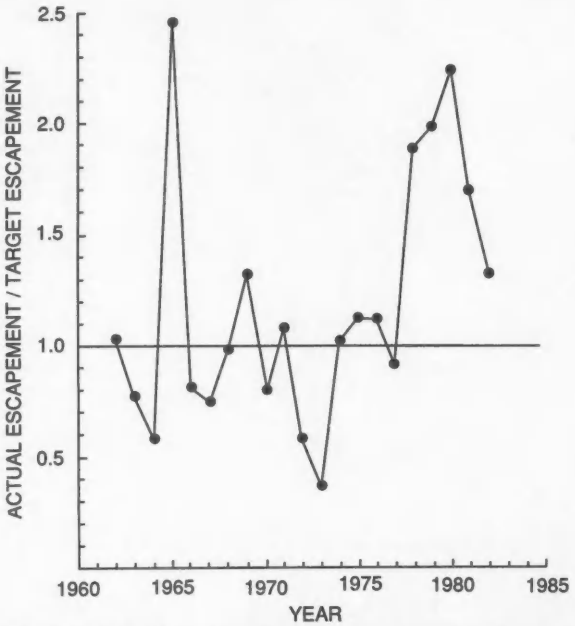

FIG. 2. Deviation of actual escapement from escapement goals for eight Bristol Bay sockeye stocks combined, 1962–82, calculated annually as the ratio of the actual escapement to the target escapement.

TABLE 2. Number of years in the 1962–82 period in which preseason forecasts of sockeye salmon recruits to Bristol Bay, Alaska, were over- or underestimated, compared with actual recruits. These frequencies are cross-tabulated by the category of actual abundance of annual escapements, whether over or under the target escapement.

Escapement relative to target	Preseason forecasts	
	Overestimated	Underestimated
Overescaped	3	9
Underescaped	8	1

Because of the inability of in-season management to completely correct for errors in preseason forecasts, we tested the null hypothesis that the direction of the deviation from target escapements is independent of the direction of error in forecasts in Bristol Bay. We used a chi-square test on data on actual escapements, target escapements, and preseason forecasts as well as actual abundances of recruits (catches plus escapement) for Bristol Bay sockeye in 1962–82, as reported in Middleton (1983) and Eggers et al. (1983). Only in these years were there data on each of these variables. We also tested the null hypothesis that the magnitude of the deviation of actual escapement from the target escapement is not related to the magnitude of the error in the preseason forecast. The stocks combined for this analysis were the Kvichak, Naknek, Egegik, Ugashik, Wood, Igushik, Nuyakuk, and Togiak River sockeye.

Results and Discussion

Actual escapements for Bristol Bay sockeye vary considerably around the target escapement (Fig. 2). The figure shows, for example, that in 1973 the number of sockeye that reached the spawning grounds was about 36% of the desired number.

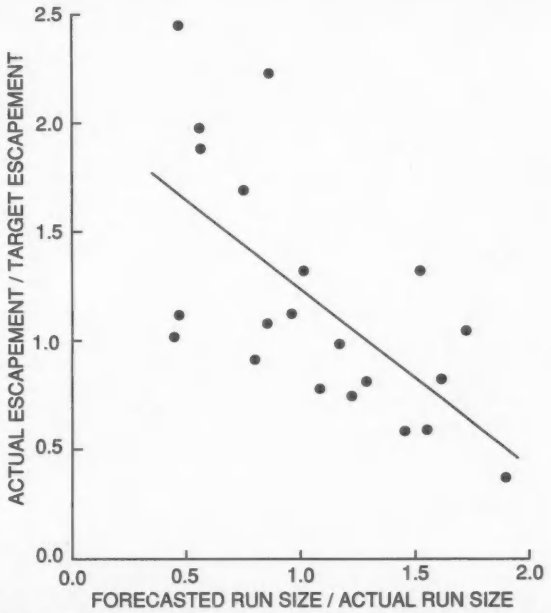

FIG. 3. Ratio of actual escapement to target escapement versus ratio of forecasted annual recruitment or run size to actual run size for Bristol Bay sockeye, 1962–82 ($r = -0.65$, $P < 0.002$), $Y = 2.06 - 0.83X$.

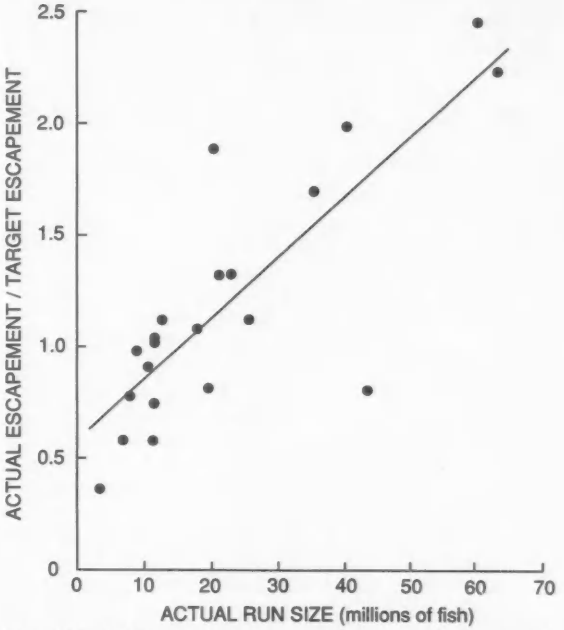

FIG. 4. Ratio of actual escapement to target escapement versus total annual recruitment or run size for Bristol Bay sockeye, 1962–82 ($r = 0.81$, $P < 0.001$), $Y = 0.58 + 0.03X$.

This failure to meet the target escapement is due in part to the inability of in-season regulations to respond quickly enough to unexpectedly large or small abundances of returns (Middleton 1983).

Direction of error

The direction of error in the preseason forecast was significantly related to whether actual escapements were above or below the target (chi-square test, $P = 0.004$, Table 2). Underescapements (too few spawners) occurred in eight of eleven years in which there were overestimates of returns of Bristol Bay sockeye (Table 2). This occurred because too much catch was allowed relative to the actual number of recruits available. Similarly, nine of ten years in which returns were underestimated resulted in overescapements of Bristol Bay sockeye (Table 2).

One mechanism that could possibly link preseason forecasts to escapements is through the in-season management approach used by ADF&G personnel. This approach entails relying strongly on the preseason forecast of abundance early in the fishing season but revising this forecast during the course of the season based on actual data (Minard and Meacham 1987). In the case of overestimates of recruits, the high preseason forecast perhaps initially made managers reluctant to make fishery openings shorter than planned, even when early in-season estimates gave preliminary indications of a smaller run than predicted. By the time it became clear that returns were indeed less abundant than forecasted, it was too late to reduce the catch enough to achieve target escapements. Preseason overestimates of returns could thus lead to actual escapements being lower than targets. The converse situation could apply to years in which returns were underestimated by the preseason forecast. In those years, more fish would return than anticipated, but this might not be realized until it was too late to harvest the fish in excess of target escapements. Thus, underestimates of returns could lead to overescapements, generating the pattern shown in Table 2.

Magnitude of error

The magnitude of deviations from the total target escapement for all Bristol Bay sockeye stocks, as measured by the ratio of the actual escapement to the target escapement, was significantly related to the magnitude of the error in preseason forecasts, as measured by the ratio of the forecasted abundance to the actual abundance (Fig. 3). When the abundance of sockeye recruits was greatly underpredicted (ratio $<<$ 1 for x-axis of Fig. 3), actual escapements tended to be much larger than the target (ratio $>$ 1). If the abundance of sockeye returns was greatly overestimated, then actual escapements were much smaller than the target escapement.

This effect was particularly pronounced for large abundances of sockeye recruits. The magnitude of deviations from the total target escapement for all Bristol Bay sockeye stocks, again as measured by the ratio of the actual escapement to the target escapement, was also significantly related to the actual run size (Fig. 4). Larger deviations occurred in years of large recruitment because it is more difficult for management to fully correct in-season for inaccuracies in preseason forecasts when runs are abundant, and hence final escapements deviate from target escapements by a greater amount than when abundances of runs are small. Recall that the inability of management to achieve target escapements is particularly pronounced for stocks such as Bristol Bay sockeye because of the narrow run timing distribution (approximately 2 wk), which does not allow much time for correction.

The long-term consequences of both over- and underestimates of abundance of sockeye recruits arise from the tendency toward under- and overescapements noted above. By definition

from the Ricker stock–recruitment curve upon which the target escapement is based, escapements over or under the optimum escapement can reduce abundances of harvestable sockeye in future years. In addition, the deviations of escapements from targets were larger when stock abundances were large.

Implications for Investment Behaviour of the Harvesting Sector

In the salmon industry, it is primarily investment behaviour that determines the extent to which costs are unusually high and potential economic rents (profits above an individual's or company's normal rate of return on investment) will be dissipated (Shaffer 1979). Evidence from other limited entry fisheries (Meany 1979; Fraser 1979) suggests that whenever there are economic rents available in such fisheries, individual fishermen will attempt to increase their own share of those rents by investing either in the purchase of a fishing permit, as would be the case for people seeking entry to the fishery, or in the application of capital to their vessels by existing permit holders. Furthermore, such investment behaviour can be triggered simply through the anticipation of economic rents over and above the cost of the investment by existing fishermen or permit buyers (Crutchfield 1979). Based on this evidence, it is reasonable to expect that the same might occur in the Bristol Bay sockeye fishery. Since the anticipation of economic rents in the fishery is likely to reflect expectations of future fish abundances, it is conceivable that preseason forecasts might also affect expectations by fishermen of economic rents, and hence influence their investment decisions. Therefore, in this part of our analysis, we tested the hypothesis that preseason forecasts have affected expectations of available rents by fishermen. This is particularly relevant because we have shown in the previous section that the current forecasting method tends to overestimate fish abundance.

It is difficult to obtain a direct measure of the economic rents available in the Bristol Bay sockeye fishery because no cost and earnings studies are available. However, economic theory suggests that an indirect measure of the potential rents available at the harvesting stage is market value of the fishing permit (Schwindt 1987). According to Schwindt (1987, p. 233), "The right to participate in an economic activity [fishing] commands a positive price only if the participant expects to earn revenues above his opportunity costs." Hence, the market value of the permit should reflect the perceived present value of future net revenues anticipated from fishing.

Methods

To test the extent to which preseason forecasts of sockeye returns to Bristol Bay may be related to the anticipation of rents, and hence investment decisions of fishermen, we analyzed data covering 1977–85, the only years in which all of our variables were measured. We performed a multiple regression, with the dependent variable being the interannual change in permit price (average permit price in year t − average permit price in year t − 1). The independent variables were (1) the forecasted change in the abundance of recruits ($\log_e$(forecasted return for year t) − $\log_e$(actual return in year t − 1)) and (2) interannual change in the landed dollar value of sockeye (landed value in year t − 1 − landed value in year t − 2). The change in landed value of sockeye over the previous period was included in the multiple regression as the best available indicator of recent profits for fishermen, which could also influence the amount that people are willing to pay for fishing permits.

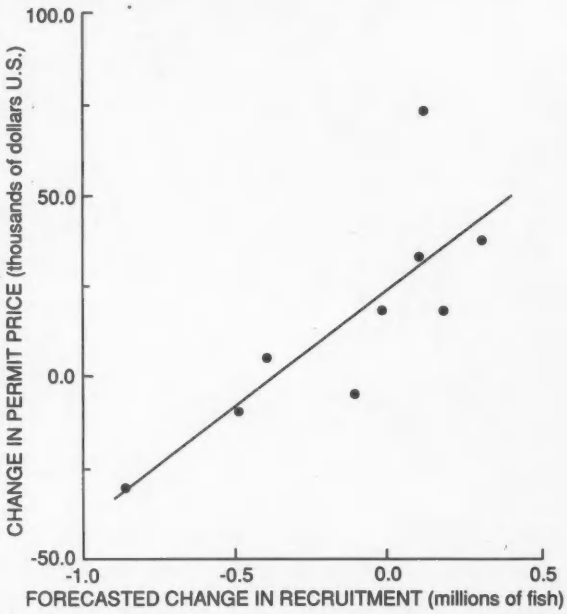

FIG. 5. Change in annual average permit price (Y) versus forecasted change in recruit abundance (forecasted compared with last year's recruits) (X) ($r = 0.80$, $P < 0.01$), $Y = 24.39 + 64.51X$.

Average permit price was the combined average price for set gill net and drift gill net permits. All monetary values were converted to constant 1985 U.S. dollars using the U.S. Consumers' Price Index (U.S. Bureau of the Census 1985). Forecasted abundance of recruits and actual abundance of recruits in the previous year were transformed using logarithms because abundance of sockeye recruits is lognormally distributed (Allen 1973; Peterman 1981).

Data on permit prices for Bristol Bay were from summary data sheets supplied by the Commercial Fisheries Entry Commission (CFEC), Juneau, Alaska. Data on permit values and the number of monetary transfers of permits are collected yearly by the CFEC and are based on active exchange markets in which permits are bought and sold (Karpoff 1983). Most of these monetary transfers take place between January and June of each year, after the preseason forecasts are issued (December) and prior to the start of the fishing season (July). During the period 1975–85, an annual average of 39 Bristol Bay set gill net permits and 72 drift gill net permits were traded (CFEC 1985). The average value of these permits in 1985 was $117,900 for the drift gill net fishery and $35,900 for the set gill net fishery. Landed values for Bristol Bay sockeye were from Muse (1986).

Results and Discussion

Results suggest that Bristol Bay gill net permit prices in the sockeye fishery are influenced by preseason forecasts. The forecasted change in the number of fish returning had the strongest partial correlation with change in the annual average permit price ($r = 0.72$, $P = 0.045$), with change in permit price increasing with increased forecasted changes in abundance. The annual change in landed value of the catch was not

a significant predictor of change in average permit price (partial $r = -0.03$, $P = 0.93$). The multiple regression equation was $P = 24.13 + 63.20F - 0.11L$, $r = 0.80$, $P = 0.046$, where $F = \log_e$(forecasted return in year t) $- \log_e$(actual return in year $t - 1$) and $L =$ landed value in year $t - 1 -$ landed value in year $t - 2$.

The correlation of forecasted change in the abundance of recruits with changes in the average permit values in Bristol Bay appeared even more strongly in a simple regression with forecasted change in abundance (Fig. 5). An increase in the average permit price occurred when forecasts anticipated an upward change in the number of recruits for a given year from the actual abundance of recruits in the previous year. This makes intuitive sense because fishermen who anticipate greater returns in the current year relative to last year will probably capitalize the value of those anticipated returns in the value of the permit.

It should be noted that the data in Fig. 5 suggest that an increase in the average permit price occurred even though there was no change in run forecast (i.e. at 0 on the x-axis). This is likely due to other factors such as increased profitability of fishing, which Karpoff (1983) showed is also correlated strongly with increases in permit prices.

Although these results do not preclude the possibility that other variables might also explain changes in permit prices, they are consistent with the hypothesis that forecasts of sockeye salmon returns have affected permit prices from 1977 to 1985 in the limited entry salmon fisheries of Bristol Bay. This is in agreement with the findings of Karpoff (1983) for Alaskan salmon fisheries in general. Because the market value of the permit reflects the profitability of the fishery, there may have been anticipation of net monetary returns, based on preliminary forecasts of sockeye salmon returns to Bristol Bay.

As mentioned earlier, this anticipation of net monetary returns can produce two distinct investment responses of fishermen. The first investment response to the anticipation of economic rents is that of purchases of permits by people currently outside the fishery. Because the price paid for permits is a reflection of expectations of available rents and is positively related to preseason forecasts, buyers run the risk of earning a low rate of return on their investment if a predicted large run of sockeye does not materialize. This, however, is not a cost to society because any rents that the buyer failed to obtain are simply transferred to the seller of that permit and out of the industry.

The second response is that of existing permit holders who are intending to remain in the fishery. Whenever there is anticipation of increased rents in the fishery, some existing permit holders will be more likely to increase the catching power of their vessels through purchase of additional equipment in an attempt to increase their share of those additional rents (Crutchfield 1979). If the anticipated increase in rents does not materialize as expected, as is the case when preseason forecasts overpredict the abundance of sockeye, then any fishermen who invested in additional equipment for that year will earn a below normal rate of return on their investment. This "overinvestment" on the part of fishermen represents a real cost to society. In conjunction with the tendency of the current forecasting method to overestimate abundance, as documented above, these investment responses could have led to overinvestment in the Bristol Bay salmon fishing fleet. This could in turn have led to decreased economic returns for individuals.

In summary, three major conclusions can be drawn regarding the biological and economic implications of incorrect preseason forecasts for Bristol Bay. First, over- or underpredictions of sockeye abundance tended to be associated with under- and overescapements, respectively. Second, the magnitude of deviations from target escapements was related to the magnitude of errors in the preseason forecast. Such deviations in escapements were more pronounced in years when the abundance of sockeye returns was large, and these deviations from target escapements reduce future yields. Improving preseason forecasts would enable managers to better achieve escapement goals and optimize long-term yields. Lastly, overpredictions can affect investment in the harvesting sector of the industry by creating expectations by fishermen of greater profits than would be realized. Improving preseason forecasts, particularly by decreasing the frequency or magnitude of overpredictions, might discourage overinvestment in the form of additional capital applied to the vessels of existing permit holders.

General Discussion

This study shows two major consequences of incorrect preseason forecasts of sockeye returns to Bristol Bay, Alaska. The first relates to the difficulty of achieving target escapements. The magnitude of deviations from escapement targets was related to the magnitude of errors in preseason forecasts. Therefore, any preseason forecast that minimizes these errors should be desirable. Since the nonlinear method using transformed abundances of sibling age-class returns was more accurate than the linear method using untransformed abundances, the nonlinear method should be preferred.

The second major consequence of incorrect preseason forecasts was that they appear to affect the anticipation by fishermen of economic rents which, according to previous experience in other fisheries, can potentially lead to overinvestment on the part of existing permit holders in the harvesting sector. Given that capital is scarce in most economies, a justification for fisheries management should be to avoid overinvestment of capital (Cunningham et al. 1985). Preseason forecasts that tend to overestimate the abundance of returning salmon could encourage this overinvestment. At least in the case of those fishermen who respond to anticipated rents by applying additional capital to their boats, improving preseason forecasts, particularly by decreasing the frequency and magnitude of overpredictions, could enable those fishermen to more closely achieve a normal rate of return. Because the nonlinear method of forecasting examined here overestimated less frequently and by a smaller magnitude than the linear method, it should again be the preferred method.

During this research, we consulted with ADF&G staff, and in 1986, they began using our nonlinear method as part of their standard forecasting technique (Fried and Yuen 1986).

Acknowledgments

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Growth, Fecundity, and Energy Stores of White Sucker (*Catostomus commersoni*) from Lakes Containing Elevated Levels of Copper and Zinc

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Munkittrick, K. R., and D. G. Dixon. 1988. Growth, fecundity, and energy stores of white sucker (*Catostomus commersoni*) from lakes containing elevated levels of copper and zinc. Can. J. Fish. Aquat. Sci. 45: 1355–1365.

White sucker (*Catostomus commersoni*) were collected from lakes with elevated levels of both copper (13–15 $\mu g \cdot L^{-1}$) and zinc (209–253 $\mu g \cdot L^{-1}$) and from control lakes. In all cases the fish reached maturity between 4 and 6 yr of age, and until 6 yr of age there were no differences in length or weight of fish collected from control and contaminated lakes. After this age, fish from contaminated sites were significantly smaller and shorter than those from control sites. In addition, female sucker from contaminated lakes failed to exhibit significant increases in either length or weight after the age of maturity. The fish from contaminated lakes also exhibited decreases in egg size and fecundity, no significant increases in fecundity with age, and an increased incidence of spawning failure. The failure of female fish to grow significantly after maturity, and the decreased energetic commitment to reproduction, suggests that the food base in the contaminated lakes was limiting the performance of the female sucker. These fish exhibited decreased muscle lipid stores, decreased serum lipid levels during the postspawning period, and an apparent decrease in visceral lipid stores during the autumn. There was no effect of collection site on body stores of liver glycogen, liver lipids, serum triglycerides, or total serum cholesterol. It is known that several major food groups were missing from the sediments of contaminated lakes and that sediments under water deeper than 5 m may be incapable of supporting macroinvertebrates. Most of the alterations in sucker growth and reproduction appear to be related to nutritional deficiencies as a result of the chronic effects of elevated sediment metals on the food base of the sucker.

Des meuniers noirs (*Catostomus commersoni*) ont été prélevés dans des lacs présentant des concentrations élevées de cuivre (13–15 $\mu g \cdot L^{-1}$) et de zinc (209–253 $\mu g \cdot L^{-1}$) et dans des lacs témoins. Dans tous les cas, les poissons ont atteint la maturité entre les âges de 4 et 6 a, et jusqu'à l'âge de 6 a il n'y avait pas de différence de longueur ni de poids chez les poissons prélevés dans les deux types de lacs. Après cet âge, les poissons des lacs pollués étaient nettement plus petits et plus courts que ceux des lacs témoins. De plus, les femelles des lacs pollués n'ont pas présenté d'augmentation notable de la longueur ni du poids après l'âge de la maturité. Les poissons des lacs pollués montraient aussi des diminutions de la taille des oeufs et de la fécondité, aucune augmentation notable de la fécondité avec l'âge, et une incidence plus forte d'échec de la reproduction. Le fait que les femelles ne se développaient pas de façon notable après la maturité, et la réduction de la quantité d'énergie consacrée à la reproduction, semblent indiquer que les ressources alimentaires des lacs pollués limitaient la performance des femelles. Ces poissons présentaient une réduction des réserves de lipides musculaires, de la teneur en lipides du sérum après le frai, et des réserves de lipides viscéraux pendant l'automne de façon notable. Le lieu de prélèvement n'avait aucun effet sur les réserves corporelles de glycogène dans le foie, de lipides dans le foie, de triglycérides dans le sérum ou de cholestérol total dans le sérum. On sait que plusieurs grands groupes alimentaires manquaient dans les sédiments des lacs pollués, et qu'il est possible que les sédiments situés à une profondeur de plus de 5 m soient incapables d'assurer la vie de macroinvertébrés. La plupart des modifications dans la croissance et la reproduction des meuniers sont en rapport avec des carences alimentaires causées par les effets chroniques des fortes concentrations de métaux dans les sédiments sur la nourriture de cette espèce.

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The detrimental effects of toxic chemicals on aquatic organisms have long been recognized. The major approach to determining and limiting the impact of aquatic toxicants has classically been the use of results from laboratory exposures to define water quality objectives for the protection of aquatic life. Unfortunately the water quality objectives approach is based on the untested and probably false assumption that an environment is protected if the objectives are met (NRCC 1985). Test conditions during laboratory studies seldom accurately reflect the environment of natural populations (Cairns 1981), and water quality objectives have seldom been validated in the field (Suter et al. 1985). Ecosystem evaluations of toxic chemical effects are costly, time consuming, and may be insufficient to provide early detection of gradual environ-

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mental deterioration (NRCC 1985). Chronic effects are seldom measurable in the field and can be dependent on environmental modifying factors (Moore and Ramamoorthy 1984) and acclimatory responses (Sprague et al. 1984).

Attempts have been made to integrate field and laboratory studies on the impacts of mercury (Saikka et al. 1978; Skei 1978), mixed metals (McFarlane and Franzin 1978; Roch et al. 1982; Klaverkamp et al. 1984; Larsson et al. 1985; Hall et al. 1987), organochlorines (Meyrle et al. 1982), and mixed contaminants (Pearce 1984; Chapman et al. 1985). The outcomes of field–laboratory comparisons are often variable and can be dependent on laboratory conditions and/or toxicant-specific whole organism responses (Boyle 1985). Furthermore, much of the testing involves the examination of biochemical parameters. Although biochemical analysis is known to be extremely valuable in situations where the contaminants are well defined (Hodson et al. 1984; Klaverkamp et al. 1984), situations involving toxicant mixtures or unknown contaminants may require the development of generalized biochemical tests (NRCC 1985; Larsson et al. 1985). Such tests are of limited value at present due to the stresses associated with capture, inadequate correlations between biochemical and whole animal effects (Neff 1985), and the requirement for the standardization of field tests with respect to biotic modifying factors (Larsson et al. 1985; NRCC 1985).

In laboratory testing, the most contaminant-sensitive aspects of a fish's life history consistently appear to be growth, reproduction, and larval survival (McKim 1977; Bresch 1982; Donaldson and Scherer 1983; Woltering 1984; Norberg and Mount 1985). This study was initiated to determine whether the toxicological impacts of mixed copper and zinc contamination in several lakes of the Manitouwadge district of northern Ontario could be detected using an ecosystem health assessment model based on the growth and reproductive capacity of indigenous populations of white sucker (*Catostomus commersoni*). The Manitouwadge chain of lakes consists of a number of small lakes (80–320 ha) near the headwaters of the Black River (approximately 49°N, 85°45′W) where copper, zinc, and silver have been actively mined since 1957 (Fig. 1). Contaminated runoff reaches the chain through both Manitouwadge and Mose lakes. The lakes are of intermediate hardness (108–112 $mg \cdot L^{-1}$ as $CaCO_3$), neutral pH (6.9–7.1), and have historically shown increased levels of copper (16 $\mu g \cdot L^{-1}$) and zinc (340–450 $\mu g \cdot L^{-1}$) (D. Hollinger, Water Resources Assessment Unit, Thunder Bay Regional Office, Ontario Ministry of the Environment, Thunder Bay, Ont. P7C 5G1, pers. comm.). The current metal levels in contaminated lakes (13–15 $\mu g\ Cu \cdot L^{-1}$, 209–253 $\mu g\ Zn \cdot L^{-1}$; Munkittrick and Dixon 1988) and the proximity of uncontaminated reference sites provided an ideal field location to test for the impacts of chronic exposure to levels of metals that approximate current water quality criteria. White sucker were chosen as the test species because they are common in the chain, respond to elevated metal levels (McFarlane and Franzin 1978; Duncan and Klaverkamp 1983; Schmitt et al. 1984), and as bottom feeders have received some attention as a possible environmental sentinel (Beamish et al. 1975; Sonstegard 1977; Swanson 1982; Klaverkamp et al. 1984; Trippel and Harvey 1987a, 1987b).

Methods

The growth and reproductive capacity of white sucker populations from control and metal-contaminated sites were assessed in terms of age, length, weight, condition factor, fecundity, and several indicators of energy status. Manitouwadge Lake (MAN) and Little Manitouwadge Lake (LMN) were selected as the metal-contaminated sites and Loken Lake (LOK) as the control site (Fig. 1). Fish were collected from MAN, LMN, and LOK with 8.8-cm (stretched) monofilament gill nets during the prespawning (PRES), postspawning (POST), and fall recrudescence (RECR) periods of 1985–87. To examine growth effects at another contaminated site, additional samples were collected from Mose Lake (MOS) during the postspawning periods of 1986 and 1987. A limited number of samples were collected from an alternative control site (Wowun Lake) during the postspawning period of 1987.

Fish were collected from the gill nets every 45–60 min, transported to shore in an adequate volume of water, and sampled as quickly as possible. During spawning (SPAW), fish were collected from the spawning beds by dip net and sampled as quickly as possible. At no time were moribund fish sampled; effects of capture stress on the physiology of the fish were assumed to be equal at all sites. Fish were anesthetized in a solution of tricaine methanesulphonate (MS222) and examined for external lesions. The weight (± 5 g) and standard length ($\pm$ 0.5 cm) of each fish were recorded. Condition factor (k) was calculated as 100(weight)/length^3. The left pectoral spine of each fish was removed at the base, dried, sectioned with a 7/0 jeweller's saw, and the annuli counted to determine age. Scales were removed from the left side of each fish above the lateral line for confirmation of age. Blood was collected via cardiac puncture using either a 4.0-mL syringe or a 5.0-mL vacuum tube, both fitted with a 21-gauge needle. Blood was clotted on ice for 6–8 h and spun for 7 min at maximum speed on a Janetzki T5 centrifuge. Sera were collected with a Pasteur pipette, placed in plastic beem capsules, frozen in liquid nitrogen, and stored at either -70 or -20°C until analysis.

The peritoneal cavity of each fish was opened and the organs were examined for abnormalities. The ovaries were removed, weighed (± 0.1 g), and preserved in 10% buffered formalin. Gonadosomatic index was calculated as 100(gonad weight)/body weight. After storage, the ovaries were washed, blotted dry, and weighed (± 0.001 g). The number of eggs in duplicate 1-g samples was determined and the results were used to estimate the total number of eggs per fish. For each fish, the diameter and weight of 10 eggs were also determined.

The viscera of the fish were removed and the liver separated from the intestine. The liver was weighed (± 0.1 g) and samples of both liver and muscle were frozen in liquid nitrogen and stored at -20°C. The liversomatic index (LSI) was calculated as 100(liver weight)/body weight. The number of parasitic acanthocephalan worms protruding through the wall of the intestine was recorded on a subjective scale with 0 being none, 1 less than 50, and 2 more than 50. Fish were also rated with respect to their visceral lipid stores during autumn (RECR) sampling periods using a subjective scale ranging from 1 to 5 (1, a <5 mm strip of lipid along the intestine with very little along the gonads; 2, a strip of lipid of approximately 5 mm along the dorsal region of both gonads and a smaller strip along the intestine; 3, lipids forming a thin layer near the body wall and extending over the posterior intestine; 4, posterior intestine covered in lipid material extending across the dorsal body wall; 5, intestine totally encased in lipids with a layer around the peritoneum).

Liver glycogen stores were determined after hydrolysis by a modified (Passonneau and Lauderdale 1974) serum glucose

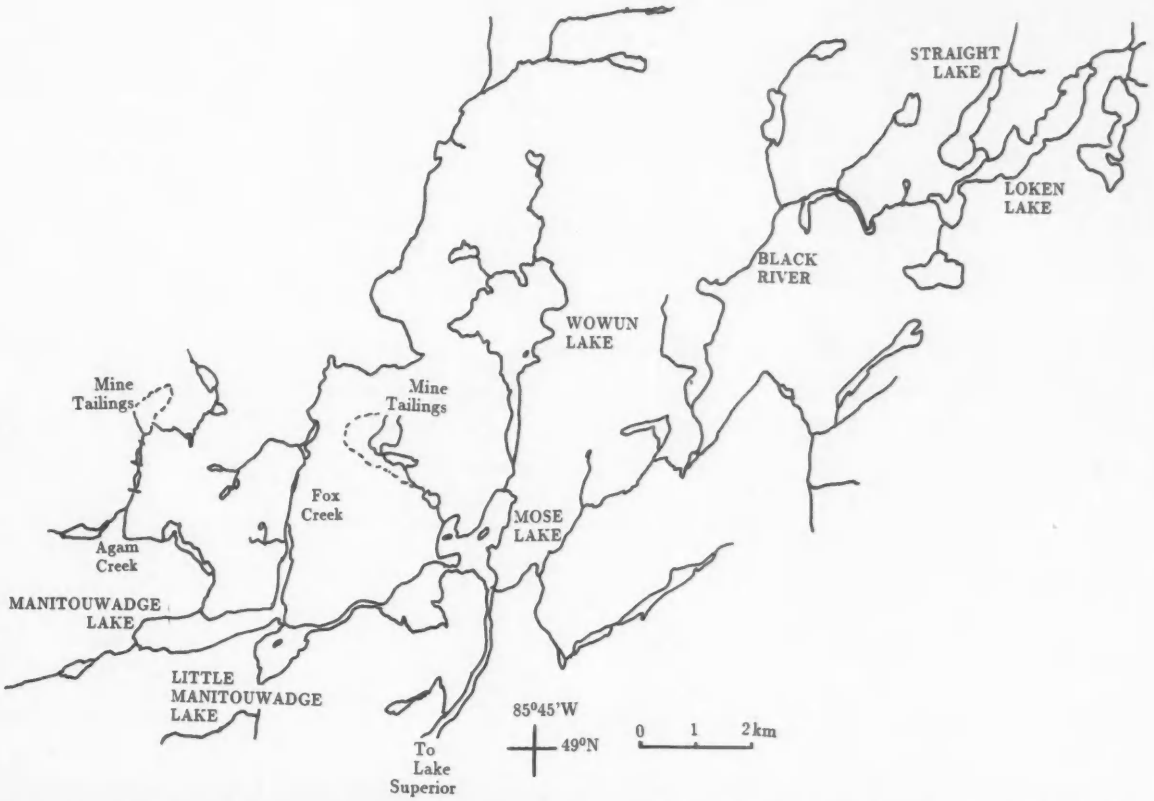

FIG. 1. Map of the study site. Manitouwadge Lake represents the elevated metal site, Little Manitouwadge Lake the low metal site, and Loken Lake the control site. Water flow is toward the south.

technique (Boehringer-Mannheim Co., Quebec). Liver and muscle total lipid estimates were performed on duplicate samples (0.1 and 1.0 g wet weight, respectively) by chloroform–methanol extraction (Bligh and Dyer 1959; Herbes and Allan 1983). Total serum lipids were measured using a spectrophotometric analysis of a phosphoric acid–vanillin reaction (Boehringer Mannheim Co.) at a wavelength of 535 nm. Serum calcium was analyzed by atomic absorption spectrophotometry using lanthanum to eliminate phosphorus interference (Beamish et al. 1975). Total serum protein was analyzed using the Biuret method (Sigma Chemical Co., St. Louis, MO) at a wavelength of 545 nm. Serum triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured with Sigma kits at wavelengths of 410, 500, and 500 nm, respectively. Alkali-labile protein-bound serum phosphorous was measured after precipitation of the serum proteins with 5 mL of cold (3°C) 10% (w/v) trichloroacetic acid. The precipitate was progressively washed with hot (80°C) ethanol and with room temperature (22°C) chloroform–ether–ethanol (1:2:2), acetone, and ether. The washed precipitate was incubated with 1 mL of 2 M NaOH for 15 min at 100°C and then neutralized with 1 mL of 2 M HCl. The resulting solution was analyzed for phosphorus content with a phosphorus kit (Sigma Chemical Co., St. Louis, MO) at 600 nm.

Data were examined for normality and homoscedasticity and compared using analysis of variance procedures available through the University of Waterloo SAS and SYSTAT packages. When main effects or interactions were significant, a conservative least square means or modified Tukey's test was used to compare treatment means (Zar 1984). Length, weight, and age regressions, as well as tests involving condition factor, serum calcium, and serum phosphorus levels, were conducted on log-transformed values. Regression coefficients were compared by *t*-test. Subjective ratings of visceral lipids, parasite load, maturity, and sex ratio were compared using nonparametric loglinear procedures for three-dimensional contingency tables. In all cases, significance levels were set at $p \leq 0.05$.

Results

Female fish were significantly longer ($p < 0.0001$) and heavier ($p = 0.0008$) than male fish regardless of site. In addition, females from control sites were significantly longer and heavier than females from contaminated sites ($p < 0.0001$) (Table 1). The only significant difference among male fish was evident during the POST period; control males were significantly longer than males from contaminated sites ($p < 0.05$). There was a marginal interaction between sex and season for condition factor ($p = 0.059$). Condition factors of male fish varied with site ($p = 0.0007$) and season ($p = 0.015$), and the condition factors of both LOK and LMN males were significantly larger than those for MAN males. Male condition factors

TABLE 1. Characteristics of white sucker collected from control (LOK) and metal-contaminated (MAN, LMN) lakes during different phases (PRES, POST, RECR) of the reproductive cycle. Values are given as means (SE, *n*). For age, means followed by the same letter are not significantly different. The statistical significance of the data for K, LSI, and GSI is given in the results section.

Lake	Male			Female			Site mean
	PRES	POST	RECR	PRES	POST	RECR	
				Age (yr)			
LOK	6.0 (0.4, 19) b	7.7 (0.7, 9) a	4.5 (0.4, 8) c	6.2 (0.4, 25) b	6.0 (0.5, 20) b	7.0 (0.3, 29) b	6.34 (0.20, 110) a
MAN	7.6 (0.3, 23) a	6.1 (0.5, 13) b	6.3 (0.8, 8) b	7.4 (0.4, 19) a	6.0 (0.4, 22) b	5.8 (0.4, 21) b	6.58 (0.18, 106) a
LMN	7.5 (0.3, 20) a	7.7 (0.3, 28) a	7.1 (0.5, 17) b	7.2 (0.2, 26) b	6.9 (0.4, 22) b	5.5 (0.4, 12) c	7.13 (0.15, 125) b
				Condition factor			
LOK	2.11 (0.04, 19)	2.15 (0.07, 10)	2.18 (0.04, 14)	2.18 (0.03, 26)	2.21 (0.05, 29)	2.09 (0.04, 33)	2.15 (0.02, 131)
MAN	1.97 (0.03, 23)	2.04 (0.06, 14)	2.05 (0.05, 14)	2.04 (0.04, 20)	2.15 (0.06, 22)	2.07 (0.04, 29)	2.05 (0.02, 122)
LMN	2.06 (0.05, 20)	2.11 (0.05, 29)	2.22 (0.05, 17)	2.15 (0.04, 25)	2.03 (0.04, 25)	2.12 (0.06, 13)	2.11 (0.02, 129)
Season mean	2.04 (0.03, 62)	2.10 (0.04, 53)	2.15 (0.03, 45)	2.13 (0.02, 71)	2.13 (0.03, 76)	2.09 (0.03, 75)	
				Liver somatic index			
LOK	1.29 (0.08, 9)	1.52 (—, 1)	0.95 (0.06, 11)	1.49 (0.07, 9)	1.66 (0.07, 9)	1.14 (0.06, 15)	1.28 (0.04, 54)
MAN	1.50 (0.08, 13)	1.56 (0.13, 6)	1.07 (0.09, 10)	1.68 (0.09, 9)	1.71 (0.12, 7)	1.31 (0.07, 19)	1.43 (0.05, 64)
LMN	1.50 (0.09, 14)	1.48 (0.07, 8)	1.11 (0.10, 9)	1.69 (0.05, 13)	1.82 (0.15, 9)	1.48 (0.15, 8)	1.52 (0.05, 61)
Season mean	1.45 (0.05, 36)	1.51 (0.07, 15)	1.04 (0.05, 30)	1.63 (0.04, 31)	1.73 (0.07, 25)	1.28 (0.05, 42)	
				Gonadosomatic index			
LOK	4.0 (0.2, 19)	1.4 (0.2, 10)	6.0 (0.3, 14)	11.7 (0.6, 18)	1.9 (0.5, 25)	4.3 (0.3, 24)	
MAN	4.4 (0.2, 22)	2.4 (0.2, 14)	6.3 (0.3, 14)	11.9 (0.4, 20)	5.1 (1.1, 22)[a]	4.6 (0.3, 29)	
LMN	4.3 (0.1, 20)	1.7 (0.1, 26)	6.2 (0.5, 17)	10.8 (0.5, 25)	1.6 (0.1, 21)	4.3 (0.3, 13)	

[a]Includes fish which failed to spawn; the mean with those fish removed was 1.9.

TABLE 2. Sex ratios (M:F) of white sucker collected from control (LOK) and metal-contaminated (MAN, LMN) lakes during different phases (PRES, POST, RECR) of the reproductive cycle. Values followed by the same letter are not significantly different.

Lake	Phase of cycle			Total
	PRES	POST	RECR	
LOK	30:34	15:41	14:33	59:108 a
MAN	24:22	21:29	14:30	59:81 a
LMN	35:26	42:30	42:13	119:69 b

during RECR were significantly higher than during PRES ($p < 0.05$). There were no effects of either site ($p = 0.079$) or season ($p = 0.564$) on the condition fctors of female fish (Table 1).

The age of fish sampled varied seasonally ($p < 0.0001$). Overall, the age of MAN fish was not significantly different from control fish, although LMN fish were slightly older ($p = 0.009$; Table 1). At LMN, male fish were significantly older than females ($p = 0.0026$), although there was no effect of sex on age at either MAN ($p = 0.54$) or LOK ($p = 0.42$). During spawning, the migration of fish to the nearshore areas altered the age distribution and sex ratio at all sites (Tables 1 and 2). The effects on age distribution varied with site; at MAN, the PRES sucker were significantly older than POST or RECR fish ($p = 0.0003$), while at LMN, both PRES and POST sucker were older than RECR ($p = 0.013$). At LOK, the POST fish were older than both the PRES and RECR fish ($p = 0.013$). Despite seasonal changes, the sex ratios of MAN and LOK fish were not significantly different ($p = 0.123$), although both were significantly different from LMN ($p < 0.001$). There was also a significant site and reproductive stage interaction on the sex ratios of the fish ($p = 0.015$).

At 4 and 5 yr of age there were no significant differences in either length or weight of sucker between the control and contaminated sites. At 7, 8, and 9 yr of age, however, control females were significantly longer and heavier than females from contaminated sites (Fig. 2a). Fish older than 10 yr of age were pooled because of their low frequency; control fish in this age group were significantly longer ($p < 0.0001$) and heavier ($p = 0.0003$) than fish from contaminated sites. Control fish exhibited significant linear relationships of age with both length and weight for male and female fish (Table 3). There were no significant effects of age on weight of female fish at any contaminated site, and only MAN females exhibited a significant relationship between length and age (Table 3). Despite this relationship, the slope was significantly lower than control fish ($p < 0.05$), and the correlation coefficient (r^2) was less than 0.10 (compared with 0.61 for controls). Effects on males were not as obvious (Fig. 2b) and regressions of age on length and weight of MAN and LMN males were not significantly different from LOK fish (Table 3).

At the control site, 40.6% (13/32) of the 4- and 5-yr-old females captured during PRES were immature. Using Lysack's formula (Trippel and Harvey 1987b), the age of maturation (Z_{age}) of LOK females was 4.8 yr compared with 4.1 yr for MAN. The estimates of reproductive life span (RLS = $\bar{x}_{age} - Z_{age}$) of females were 2.33 yr for LOK and 2.47 yr for MAN. MAN sucker exhibited an increased incidence of spawning failure among females between the ages of 4 and 7 (7/22) compared with control fish (3/40) ($p = 0.033$). All control females which

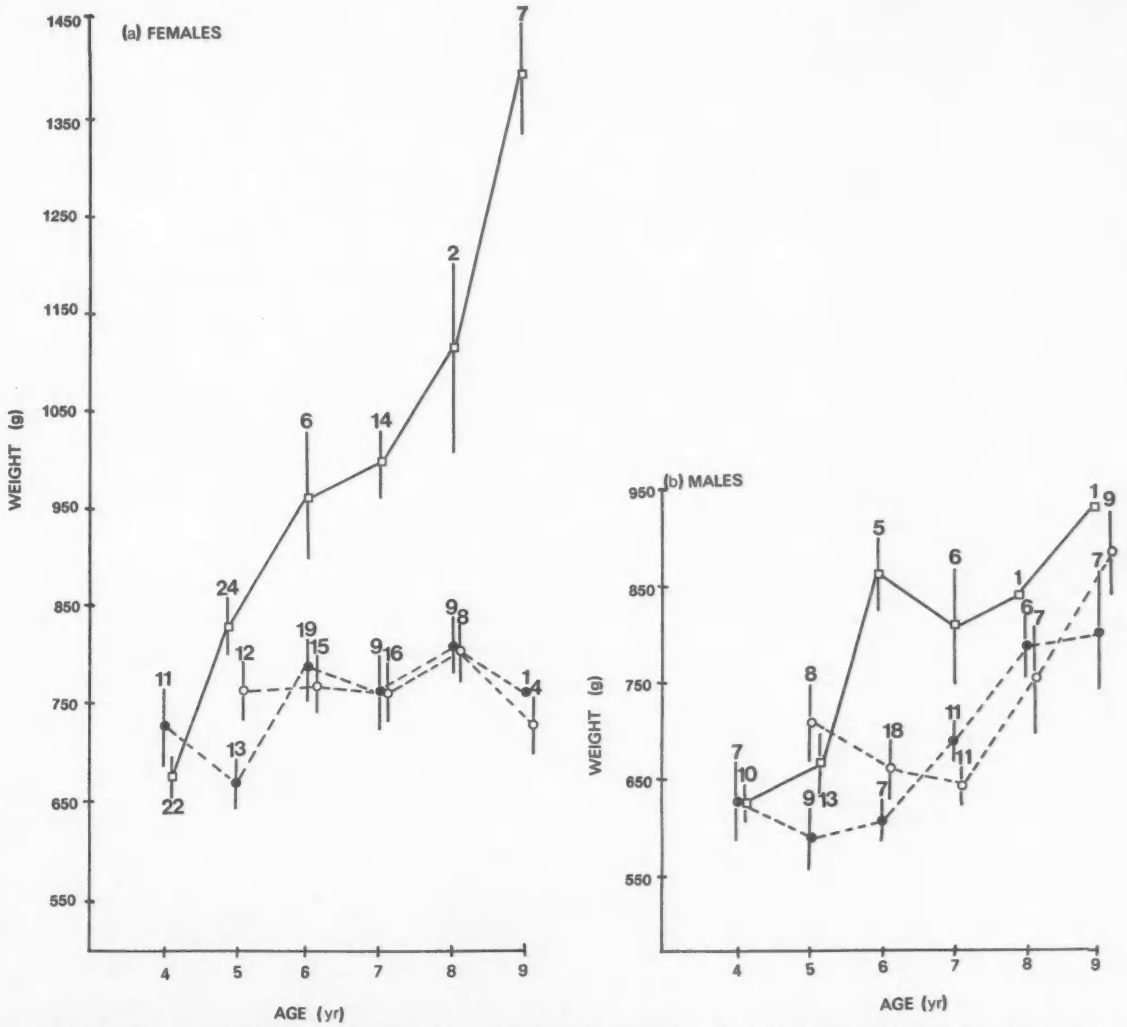

FIG. 2. Weight and age of (a) female and (b) male white sucker from Manitouwadge Lake (solid circles), Little Manitouwadge Lake (open circles), and the control site (open squares). Values are given as means with two standard errors and the sample size shown.

failed to spawn were aged 5 yr, while at MAN the average was 5.9 yr. One additional MAN female of age 10 yr also failed to spawn. There was a highly significant effect of season on the gonadosomatic index (GSI) ($p < 0.0001$) (Table 1). There was no significant effect of site on the GSI of either male ($p = 0.299$) or female fish ($p = 0.355$), if females fish which failed to spawn were removed from the comparison. There was also no difference detected in the stages of egg development between sites (K. R. Munkittrick and D. G. Dixon, unpubl. data).

The mean ($\pm$ SE) weight of control eggs (18.7 $\pm$ 0.3 mg) was significantly greater than the mean for eggs from the MAN site (17.2 $\pm$ 0.3 mg) ($p < 0.05$). Control fish also had significantly more eggs ($\bar{x}$, SE, n: 31 200, 2619, 19) than fish from either MAN (22 635, 746, 26) or LMN (21 863, 958, 22) ($p < 0.0001$). The effect of site on fecundity was significant in all relationships of fecundity using length, weight, and/or age as covariates. Fecundity was significantly correlated with both length and weight at all sites, although the correlations were significantly stronger at the control site (Table 4). Fecundity was also highly correlated with the age of control fish ($p < 0.001$), but exhibited no such relationship at either contaminated site (Table 4).

There were significant effects of sex, site, and season on the liversomatic index (LSI). Female fish had a larger LSI than males ($p < 0.0001$), and the LSI of control fish was significantly smaller than either LMN or MAN fish ($p = 0.0056$) (Table 1). RECR LSIs were significantly smaller than either PRES or POST values ($p < 0.0001$). Fish from the control site exhibited a significantly higher prevalence and intensity of infection with intestinal acanthocephalans than found at either contaminated site ($p < 0.0001$; Table 5). There was no effect of sex on parasite burden ($p = 0.56$).

At the contaminated site, female fish exhibited obvious effects on growth and fecundity, suggesting that differences in energy stores or lipid metabolism could be evident. Estimates

TABLE 3. Regressions for log length and log weight against age of white sucker from metal-contaminated (MAN, LMN, MOS) and control (LOK) lakes. For slopes and intercepts, values within columns followed by the same letter are not significantly different. NS = not significant.

Sex	Lake	Slope	$p(b=0)$	Intercept	p(int = 0)	r^2	n
			Log length				
M	MAN	0.0101 a	<0.0001	1.444 b	<0.0001	0.41	47
M	LMN	0.0095 a	0.0001	1.444 b	<0.0001	0.25	52
M	MOS	0.0063	0.52	1.470	NS	0.06	9
M	LOK	0.0111 a	<0.0001	1.446 b	<0.0001	0.39	37
F	MAN	0.0056 b	0.0123	1.485 b	<0.0001	0.98	63
F	LMN	−0.0006	0.79	1.520	NS	0.001	53
F	MOS	0.0044	0.44	1.510	NS	0.041	17
F	LOK	0.0202 c	<0.0001	1.420 b	<0.0001	0.610	76
			Log weight				
M	MAN	0.0275 a	<0.0001	2.65 b	<0.0001	0.37	47
M	LMN	0.0218 a	0.0001	2.70 b	<0.0001	0.14	52
M	MOS	0.0161	0.399	2.74	NS	0.10	9
M	LOK	0.0367 a	<0.0001	2.65 b	<0.0001	0.39	37
F	MAN	0.0115 b	0.067	2.81 a	<0.0001	0.054	63
F	LMN	−0.0033	0.66	2.91	NS	0.004	53
F	MOS	0.017	0.285	2.79	NS	0.076	17
F	LOK	0.0587 c	<0.0001	2.60 b	<0.0001	0.64	76

TABLE 4. Coefficients for relationships for fecundity (total number of eggs) with age, length, and weight of white sucker from control (LOK) and metal-contaminated (MAN, LMN) lakes. NS = not significant.

Lake	Slope	$p(b = 0)$	Intercept	p(int = 0)	r^2	n
		Age (yr)				
LOK	4602.4	0.0001	−47.7	<0.001	0.660	19
LMN	1183.8	0.226	13 310	NS	0.072	22
MAN	741.8	0.093	17 613	NS	0.118	25
		Length (cm)				
LOK	2259.0	0.0001	−49 708	<0.001	0.880	19
LMN	1526.9	0.0003	−29 565	<0.001	0.489	22
MAN	1092.4	0.0005	−13 602	<0.001	0.399	26
		Weight (g)				
LOK	25.92	0.0001	3 454	<0.001	0.951	19
LMN	20.88	0.0001	4 679	<0.001	0.641	22
MAN	22.68	0.0001	5 219	<0.001	0.510	26

TABLE 5. Subjective estimates of visceral lipid stores and the incidence of parasitic acanthocephalans for white sucker from control (LOK) and metal-contaminated (MAN, MOS) lakes. Values are given as means (SE, n). Values within columns followed by the same letter are not significantly different. All estimates were made during the RECR period of 1986.

Lake	Male	Female	Mean	Prevalence (%)
		Lipids		
MAN	1.7 (0.3, 6)	2.3 (0.2, 8)	2.0 (0.2, 14)	—
MOS	1.6 (0.2, 10)	2.3 (0.3, 10)	1.9 (0.8, 20)	—
LOK	1.6 (0.2, 6)	3.1 (0.5, 8)	2.7 (0.4, 14)	—
		Parasites		
MAN	0.85 (0.40, 6)	0.25 (0.16, 8)	0.5 (0.2, 14) a	35 a
MOS	0.60 (0.33, 10)	1.10 (0.48, 10)	0.9 (0.3, 20) a	35 a
LOK	3.67 (0.33, 6)	3.13 (0.51, 8)	3.4 (0.3, 14) b	93 b

TABLE 6. Levels of liver glycogen, liver lipids, and muscle lipids of white sucker collected from control (LOK) and metal-contaminated (MAN) lakes during different phases (PRES, POST, RECR) of the reproductive cycle. Values are given as means (SE, *n*). Values followed by the same letter within a sex are not significantly different.

		Liver glycogen ($mg \cdot g^{-1}$)		Liver lipids ($mg \cdot g^{-1}$)		Muscle lipids ($mg \cdot g^{-1}$)	
Sex	Phase	MAN	LOK	MAN	LOK	MAN	LOK
M	PRES	72.1 (3.2, 8) a	45.5 (10.4, 7) a	63.0 (8.0, 9) a	47.1 (10.0, 3) a	—	—
M	POST	34.7 (8.8, 8) b	38.4 (7.8, 5) b	51.0 (6.9, 7) a,b	46.7 (5.8, 7) a,b	—	—
M	RECR	23.8 (4.8, 7) b	28.1 (4.9, 7) b	41.7 (3.7, 12) b	33.1 (2.1, 13) b	7.4 (0.4, 9) a	7.1 (0.3, 5) a
F	PRES	22.0 (5.6, 9) a	23.9 (6.0, 10) a	27.5 (3.7, 9) a	33.2 (4.7, 8) a	—	—
F	POST	41.3 (5.5, 10) b	37.8 (7.0, 12) b	41.4 (8.9, 9) b	49.3 (5.6, 12) b	6.2 (0.5, 8) a	8.9 (1.0, 8) b
F	RECR	18.1 (4.9, 11) a	26.4 (3.3, 10) a	33.0 (2.2, 20) a	32.9 (1.4, 21) a	6.3 (0.6, 8) a	9.1 (1.5, 5) b

of total muscle lipids during RECR indicated no effect of sex ($p = 0.51$) and a marginal interaction between sex and site ($p = 0.057$). Control females exhibited significantly higher muscle lipid levels than did MAN females ($p = 0.004$), although there was no difference between males ($p = 0.90$) (Table 6). The differences in female muscle lipid levels were consistent during different seasons ($p = 0.92$), and MAN levels were significantly lower than controls during both the POST and RECR periods ($p = 0.039$). Visual observations of visceral lipid stores during the fall collection period suggested a decrease in lipid stores at contaminated sites entering the overwintering period. However, statistical analysis could not detect an effect of site ($p = 0.56$), even though only 1/17 females at the contaminated site reached a value of 3 on the subjective scale. Analysis was complicated by an interaction between the sex of fish and the visceral fat estimate ($p = 0.02$). Attempts to accurately weigh the deposits under field conditions were too variable to be of use.

Examinations of other quantitative estimates of lipid status failed to detect many differences at the contaminated sites. There were significant interactions of sex and season for measurements of liver lipids ($p = 0.012$), liver glycogen ($p < 0.0001$), serum lipids ($p = 0.0001$), serum triglycerides ($p = 0.0004$), and total cholesterol ($p = 0.0012$). Due to the interactions with sex of fish, males and females were analyzed separately. In male fish there was no effect of site on liver lipids ($p = 0.06$), liver glycogen ($p = 0.297$), serum lipids ($p = 0.31$) serum triglycerides ($p = 0.47$), or total cholesterol ($p = 0.35$), but seasonal changes were found in all cases except cholesterol ($p = 0.445$). PRES levels of liver lipids were significantly higher than RECR ($p = 0.010$), PRES liver glycogen was elevated over both POST and RECR levels ($p = 0.0001$), PRES triglycerides were higher than SPAW or RECR ($p = 0.008$), and SPAW levels of serum lipids were higher than either POST or RECR ($p = 0.0006$) (Tables 6 and 7).

In female fish, total cholesterol levels were significantly higher at MAN than at LOK ($p = 0.007$). Total serum lipids exhibited a marginal interaction between site and season ($p = 0.06$) due to a significant POST diference between sites ($p = 0.0097$). No effects of site were detected on liver lipids ($p = 0.188$), liver glycogen ($p = 0.63$), or serum triglycerides ($p = 0.26$). POST levels of liver lipids and glycogen were elevated over PRES and RECR ($p = 0.002, p = 0.004$). Similarly, serum triglyceride levels during POST were elevated over SPAW and PRES periods. Total serum cholesterol levels during RECR were higher than all other seasons ($p < 0.0001$).

HDL cholesterol levels exhibited a marginal interaction between sex, site, and season ($p = 0.056$) (Table 7). In MAN males, PRES levels were significantly less than SPAW ($p = 0.016$), and RECR levels of LOK males were significantly higher than POST ($p = 0.015$). In females, there was no effect of season ($p = 0.058$).

There were no significant effects of sex ($p = 0.78$), site ($p = 0.78$), or season ($p = 0.69$) on the levels of total serum protein (Table 8). At LOK there was no effect of sex ($p = 0.09$) or season (PRES, SPAW, RECR; $p = 0.40$) on serum calcium levels. MAN levels of serum calcium were significantly higher than LOK ($p = 0.02$), and values for females were higher than those for males ($p = 0.007$), although there was no seasonal difference between PRES and RECR ($p = 0.71$) (Table 8). There was no effect of site on alkali-labile protein-bound serum phosphorus ($p = 0.17$). There were, however, significant ($p = 0.005$) seasonal effects; SPAW levels were significantly lower than PRES levels.

Discussion

The lack of significant relationships between weight and age for female fish at contaminated sites suggests that these fish did not show significant growth during the period of examination. The size and age data demonstrate that control females were longer and heavier than females from contaminated lakes at all ages after sexual maturation. Exposure to metals has been associated with decreased growth (Lett et al. 1976; Dixon and Sprague 1981; Seim et al. 1984; Collvin 1985), with a transient decrease usually occurring at the beginning of exposure (Sinley et al. 1974; Lett et al. 1976). This initial decrease in growth has been attributed to the synthesis of protective proteins (Roch et al. 1982; Bradley et al. 1985) and to initial decreases in appetite (Bengtsson 1974; Lett et al. 1976). Although acclimation of growth may be slow (Collvin 1985), growth rates return to normal (Buckley et al. 1982) and eventually may surpass the growth of controls (Pickering 1968; McKim and Benoit 1974; McFarlane and Franzin 1978), possibly due to increased food intake (Farmer et al. 1979). These changes are not consistent with those reported here. In this study the growth differences at contaminated sites became more apparent as the fish aged. The effect on females was greater than males, and the correlation between decreased size and sexual maturation of females at the contaminated sites suggests that the fish were unable to meet the energetic demands of both growth and reproduction after sexual maturity. Relative to controls, females from contaminated sites exhibited decreased serum lipid levels during the postspawning period and decreased muscle lipid levels during the period of gonadal growth. Males did not exhibit significant differences in these characteristics. Visual observation of visceral lipid deposits before winter were also suggestive of

TABLE 7. Levels of total lipid, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in blood sera of white sucker collected from control (LOK) and metal-contaminated (MAN) lakes during different phases (PRES, SPAW, POST, RECR) of the reproductive cycle. Values are given as means (SE, *n*). Within sex, values followed by the same letter are not significantly different. Uppercase letters are restricted within site. An asterisk designates a significant difference between sites.

Sex	Phase	Total lipids ($g \cdot L^{-1}$)		Total cholesterol ($mg \cdot dL^{-1}$)		HDL cholesterol ($mg \cdot dL^{-1}$)		Triglycerides ($mg \cdot dL^{-1}$)	
		MAN	LOK	MAN	LOK	MAN	LOK	MAN	LOK
M	PRES	23.8 (5.5, 6) a,b	23.0 (3.9, 6) a,b	361.2 (34.3, 5) a	347.7 (16.9, 5) a	51.0 (5.5, 5) a	57.0 (1.3, 4) A,B	926 (107, 5) a	1383 (236, 5) a
M	SPAW	32.6 (2.4, 6) b	27.5 (3.1, 6) b	381.4 (29.9, 6) a	355.4 (14.2, 6) a	71.9 (2.0, 5) b	63.0 (3.8, 5) A,B	454 (110, 5) b	755 (190, 5) b
M	POST	22.9 (3.2, 6) a	20.2 (1.6, 6) a	329.7 (17.9, 6) a	302.7 (62.2, 6) a	62.6 (2.9, 5) a,b	47.8 (4.7, 4) B	851 (185, 4) a,b	605 (156, 4) a
M	RECR	17.0 (1.7, 6) a	17.1 (1.4, 6) a	338.3 (33.9, 6) a	300.3 (59.6, 6) a	62.2 (4.6, 5) a,b	67.2 (3.9, 5) A	618 (176, 5) b	707 (113, 4) b
F	PRES	16.8 (4.3, 6) a	19.9 (2.2, 6) a	294.7 (28.8, 6) a	255.7 (36.6, 6) A	46.6 (4.2, 5) a	46.0 (4.1, 5) a	548 (116, 5) a,c	840 (256, 5) a
F	SPAW	20.2 (2.4, 6) a	15.6 (1.6, 6) a	308.0 (21.0, 6) a	216.9 (28.3, 6) A	59.4 (2.7, 5) a	56.7 (6.1, 4) a	667 (373, 4) a	254 (77, 5) a
F	POST	18.3 (3.4, 6)*	28.6 (3.1, 6)*	314.2 (29.3, 6) a	257.0 (30.1, 6) A	49.8 (4.2, 5) a	54.0 (8.6, 5) a	1678 (294, 5) b,c	1413 (250, 5) c
F	RECR	22.1 (3.3, 6) a	23.3 (1.5, 6) a	414.0 (12.6, 6) b	381.0 (24.3, 5) B	67.4 (9.0, 4) a	55.0 (4.4, 5) a	894 (152, 5) c	653 (143, 5) c

decreased energy stores at the contaminated sites. Again the effect on females was greater than males, an observation consistent with the theory that females attribute a higher proportion of their energy stores to reproduction.

A large proportion of young females failed to release their eggs at the contaminated sites. It may not be unusual to find females with mature ovaries after the completion of the normal spawning period; they have been recorded in Lake Superior (C. Portt, C. Portt and Assoc., 56 Waterloo Ave., Guelph, Ont. N1H 3H5, pers. comm.) and the phenomenon was evident at the control site in this study. Consequently, the effects cannot be attributed to the effects of chronic metal exposure alone. Although the age of the fish which failed to spawn at the control site suggests that they were attempting to spawn for the first time, spawning failures at the contaminated site did not appear to be restricted to first-time spawners. McFarlane and Frazin (1978) reported that some sucker exposed to a mixture of zinc, copper, and cadmium failed to release eggs, but this was not evident at their control site and no mention was made of an age-associated effect. It does not appear that this phenomenon is related to the apparent 2-yr spawning cycle in some northern fishes (Dutil 1986) because the fish had fully mature ovaries at the time of failure.

At the contaminated sites, no effects were detected on gonadal development measured morphologically (GSI) or histologically (unpubl. data). Furthermore, there was no detectable impact on the fertilization performance of sucker at the contaminated site, either at the laboratory or in situ (Munkittrick and Dixon 1988). However, eggs from contaminated sites which were incubated in clean water and reared in the laboratory resulted in smaller larvae that developed faster but did not grow as well as control larvae (Munkittrick and Dixon 1988). In addition, eggs incubated in the contaminated sites exhibited a further decrease in size, increased larvae deformities, and larvae did not perform as well when exposed to copper or zinc in the laboratory (K. R. Munkittrick, unpubl. data).

Fecundity and egg size were significantly decreased at the contaminated sites, suggesting that less energy was committed to reproduction at those sites. Metal exposure can decrease brood size and larval size in the guppy (*Poecilia reticulata*) (Uviovo and Beatty 1979) and can cause a decreased fecundity in fathead minnow (*Pimephales promelas*) (Brungs 1969). However, McFarlane and Franzin (1978) reported an increased fecundity in metal-exposed white sucker. Although fecundity usually increases with fish length (Wootton 1979), fecundity at the contaminated sites exhibited no relationship with age, a reflection of the failure of these fish to exhibit significant increases in size after sexual maturity. In addition to decreased fecundity and egg size, larvae hatched from eggs collected at the contaminated sites exhibited a 30% decrease in survival time when not fed (Munkittrick and Dixon 1987). The decreased energetic commitment to reproduction is again suggestive of nutritional limitations.

Both the quantity and quality of food can affect bioenergetics, the amount of energy for growth (Dixon and Hilton 1981), and the chronic toxicity of metals (Dixon and Hilton 1985). Furthermore, there is a direct association of food supply with both the growth of sucker (Trippel and Harvey 1987a) and fecundity in salmonids (Scott 1962; Bagenal 1969). When food consumption levels are unfavourable, egg production takes place at the expense of decreased weight and somatic energy content in some species (Hislop et al. 1978; Wootton 1979). Although food restriction and age of maturity can affect both

TABLE 8. Levels of protein, calcium, and alkali-labile protein-bound phosphorus in blood sera of white sucker collected from control (LOK) and metal-contaminated (MAN) lakes during different phases (PRES, SPAW, POST, RECR) of the reproductive cycle. Values are given as means (SE, *n*). Values followed by the same letter are not significantly different.

		Serum protein ($mg \cdot mL^{-1}$)		Serum Ca ($mg \cdot dL^{-1}$)		Serum P ($mg \cdot dL^{-1}$)	
Sex	Phase	MAN	LOK	MAN	LOK	MAN	LOK
M	PRES	38.0 (2.2, 6) a	42.3 (2.4, 6) a	17.2 (3.1, 10) c	13.6 (4.1, 8) a	—	—
M	SPAW	41.1 (2.7, 6) a	43.9 (4.3, 6) a	—	15.1 (3.5, 8) a	—	—
M	POST	39.6 (0.7, 6) a	41.2 (3.7, 6) a	—	—	—	—
M	RECR	37.6 (1.4, 6) a	38.2 (2.0, 6) a	11.4 (1.6, 10) c	9.1 (0.5, 8) a	—	—
F	PRES	43.0 (3.4, 6) a	38.6 (1.6, 6) a	20.9 (2.6, 10) b	12.6 (2.4, 8) a	16.6 (3.4, 8)	16.3 (3.7, 7)
F	SPAW	41.0 (2.3, 6) a	40.8 (3.8, 6) a	—	16.5 (3.2, 8) a	4.7 (1.7, 6)	10.8 (3.1, 7)
F	POST	53.4 (2.7, 6) a	38.8 (1.7, 6) a	—	13.7 (3.1, 6) a	16.1 (5.6, 5)	24.0 (3.4, 8)
F	RECR	42.3 (3.3, 6) a	41.1 (2.2, 6) a	16.6 (1.5, 10) b	17.6 (1.7, 8) a	10.2 (1.6, 8)	8.1 (1.8,8)

fecundity and growth, sucker at the contaminated sites did not show significant differences from controls until sexual maturation. This suggests that the nutritional deficiency is marginal and that the fish can grow normally until faced with the added demands of reproduction. This is further supported by the absence of a change in liver glycogen stores or liver lipid levels in the fish from contaminated lakes.

The increased liver size at the contaminated sites is consistent with increased metabolic activity (Larsson et al. 1985). Because the fish were capable of achieving normal liver glycogen and lipid levels, and because the average size of fish was not different until after maturation, any hepatic impairment appears to be negligible. Liver metal burdens were elevated at the contaminated sites, as were the levels in kidney, gills, and ovaries (K. R. Munkittrick and D. G. Dixon, unpubl. data), but again, metabolic impairments were not obvious. The decreased incidence of parasitic infections has been previously reported for sucker collected from contaminated sites (Swanson 1982). It is unknown whether the decreased success of parasites in contaminated environments is associated with a decreased tolerance of the adults or the intermediate stages.

Seasonal changes in serum levels of protein, lipids, triglycerides, calcium, and phosphorus were examined for indications of energy status and changes associated with gonadal development. Serum protein, total lipids, and serum triglyceride levels were within the range of values reported by Lockhart and Metner (1984) and Hille (1982). Although changes in serum protein and total lipids were not suggestive of increased production during vitellogenesis, levels of triglycerides and total cholesterols were. Furthermore, alkali-labile protein-bound phosphorus levels in the serum of female fish were significantly higher during the prespawning period than during spawning. Serum calcium levels in female fish were elevated over those in males during RECR at both sites. These changes appeared to be related more to a decline in male calcium levels than to an increase in females. Seasonal changes in serum calcium were not evident in females collected at the control site. Measurements of serum calcium, protein, and phosphoprotein have been used as simple indicators of vitellogenesis (Nagler et al. 1987). Serum calcium levels have been shown to follow seasonal patterns of 17-β-estradiol and testosterone in salmonids (Duston and Bromage 1986) and serum protein levels are thought to decline before vitellogenesis (van Bohemen et al. 1981; Hille 1982). Seasonal changes in serum calcium are not always apparent during the reproductive cycle (Bjornsson et al. 1986). In white sucker, the failure to release eggs as a result of acidification has been related to decreased serum calcium levels (Beamish et al. 1975), although the levels of calcium were similar to those observed in this study during successful reproduction of sucker.

Sucker at the contaminated site are known to be feeding on different organisms (unpubl. data) than those at the control site, and the invertebrate fauna is different at control and contaminatfored sites within the chain (Pugh and Maki 1986). The metal-contaminated lakes do not appear to be capable of supporting invertebrate fauna below 5–7 m of depth (German 1971; Pugh and Maki 1986) and several major food groups are absent or in low density in shallow areas at contaminated sites (MAN and MOS), including clams, snails, and large species of Trichoptera and Ephemeroptera, while species of Chironomids and Tubificids dominate (K. R. Munkittrick and D. G. Dixon, unpubl. data). Metal levels in the sediments of MAN average 102 mg $Cu \cdot kg^{-1}$ and 1150 mg $Zn \cdot kg^{-1}$ and metal levels in the stomach contents of suckers exceed 1600 mg $Zn \cdot kg^{-1}$ (K. R. Munkittrick and D. G. Dixon, unpubl. data).

Van Loon and Beamish (1977) did not detect any effect of exposure to 90 μg $Zn \cdot L^{-1}$ and 10 μg $Cu \cdot L^{-1}$ on the growth, year-class strength, or abundance of fish. However, some adverse effects were found in lakes with levels of zinc between 200 and 1000 $\mu g \cdot L^{-1}$ (Van Loon and Beamish 1977). Removal of fish from a population as a result of contaminant impact should have the same effect on the population as removal by fishing. Such changes would be expected to induce density-dependent compensation, and a relative increase in food resources would be expected to result in increased growth, earlier maturation, and increased fecundity. McFarlane and Franzin (1978) reported increased growth and fecundity, earlier maturation, decreased spawning success, decreased larval and egg survival, smaller egg size, and decreased longevity in sucker exposed to a mixture of metals. Sucker in this study exhibited decreased growth and fecundity despite the presence of copper and zinc levels similar to the lakes examined by McFarlane and Franzin (1978). The absence of density-dependent compensation in Manitouwadge sucker suggests that either mortalities associated with the metals are insufficient to induce compensatory mechanisms or that the metals have altered the environment so that the carrying capacity of the system has been reduced to a new level, consistent with a decreased population size. It seems likely that most of the changes evident in these populations of sucker are a result of an indirect effect of the metals on the food items of the fish in the contaminated lakes. The levels of copper and zinc in both the water and sediment of Manitouwadge Lake have declined substantially since the late 1960's. German (1971) reported waterborne copper and

zinc levels in Manitouwadge Lake of 26 and 400 $\mu g \cdot L^{-1}$ and sediment concentrations of 700 and 4400 $mg \cdot kg^{-1}$ (German 1971). Presumably the contamination of lakes by atmospheric deposition (McFarlane and Franzin 1978) would not involve levels of sediment contamination comparable with past records at the Manitouwadge site. Consequently, invertebrate populations may have been spared the eradication associated with sediment contamination in the Manitouwadge lakes.

In summary, changes in reproduction and growth were detected using an environmental health assessment approach. Female fish from the contaminated sites exhibited decreased size after maturation, decreased muscle lipid levels, and decreased total serum lipid levels during the postspawning period. The lack of obvious effects on males is consistent with a hypothesis that food is limiting because females require more energy to sustain their reproductive efforts. Fish collected at the contaminated sites did not differ in sex ratio or age at maturity, but females exhibited increased spawning failure, decreased fecundity, and decreased egg size. The decreased energetic commitment of females to reproduction and decreased growth after sexual maturity are consistent with findings of decreased food availability associated with increased sediment loading of metals.

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A Field Experiment to Test the Importance of Estuaries for Chinook Salmon (*Oncorhynchus tshawytscha*) Survival: Short-Term Results

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Macdonald, J. S., C. D. Levings, C. D. McAllister, U. H. M. Fagerlund, and J. R. McBride. 1988. A field experiment to test the importance of estuaries for chinook salmon (*Oncorhynchus tshawytscha*) survival: short-term results. Can. J. Fish. Aquat. Sci. 45: 1366–1377.

In late April of 1983, 1984, and 1985, 140 000 marked chinook salmon (*Oncorhynchus tshawytscha*) smolts (2–4 g) were transported by helicopter from Quinsam Hatchery to four release sites near Campbell River, B.C. (river, estuarine, transition, and marine), in an experiment to test the importance of estuarine residency to chinook survival. At the marine site, fish were released directly into seawater. These fish had high cortisol levels and larger interrenal nuclear diameters than those at the estuarine site, indicating a transitory stress response to seawater exposure. Nevertheless, there was little direct mortality due to stress or osmoregulatory shock at any of the release sites. Marine-released fish were exposed to more bird and fish predators. Mortality of caged chinook was higher at the marine location than at all other sites despite seawater challenge tests indicating that the chinook were smolted and "ready for sea." Beach seine data obtained biweekly for 4 mo after the releases showed that fish released directly into marine waters rarely dispersed to the Campbell River estuary. Fish released immediately adjacent to the mouth of the estuary (transition zone) had the widest immediate dispersal pattern, with many of them returning to the estuary. Estuarine zone fish displayed the most restricted distribution. Fish released to the river and estuary remained in the sampling area for a longer period (34–47 d) than those released in the marine or transition zone (20–23 d).

À la fin des mois d'avril de 1983, 1984 et 1985, on a transporté par hélicoptère 140 000 jeunes saumons quinnats (*Oncorhynchus tshawytscha*) marqués (2–4 g) provenant de la piscifacture Quinsam à quatre sites de remise à l'eau près de Campbell River (C.-B.) (rivière, milieu estuarien, milieu de transition et milieu marin), dans le cadre d'une expérience visant à vérifier l'importance du séjour estuarien pour la survie du saumon quinnat. Au site marin, on a libéré les poissons directement dans l'eau de mer. Ces poissons présentaient des niveaux de cortisol élevés et des diamètres nucléaires interrénaux plus importants que ceux se trouvant au site estuarien, ce qui indique une réaction de stress transitoire à l'exposition à l'eau salée. Néanmoins, il y a eu peu de mortalité directe attribuable au stress ou au choc de régulation osmotique à chacun des sites de remise à l'eau. Les poissons lâchés en mer ont été exposés à un plus grand nombre de poissons et d'oiseaux prédateurs. La mortalité des saumons quinnats gardés en cage a été plus élevée au site marin qu'à tous les autres sites en dépit de tests de provocation à l'eau de mer montrant que les saumons quinnats avaient subi la smoltification et étaient « prêts à se rendre en mer ». Les données de capture à la senne de rivage obtenues deux fois par semaine pendant 4 mo après les remises à l'eau ont montré que les poissons lâchés directement en mer se dispersaient rarement jusqu'à l'estuaire de la rivière Campbell. Les poissons libérés dans le voisinage immédiat de l'embouchure de l'estuaire (zone de transition) présentaient la dispersion immédiate la plus étendue, un grand nombre d'entre eux revenant à l'estuaire. La distribution la plus limitée a été observée chez les poissons de la zone estuarienne. Les poissons libérés dans la rivière et dans l'estuaire sont demeurés dans le secteur d'échantillonnage plus longtemps (34–47 d) que ceux remis à l'eau dans le milieu marin ou dans la zone de transition (20–23 d).

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The use of Pacific coast estuaries by juvenile salmonids for feeding, physiological adaptation, and predator avoidance has been documented recently (Healey 1980; Myers and Horton 1982; Wolf et al. 1983; Iwata and Komatsu 1984). However, controversy exists as to how important estuarine residence is to the survival of juvenile Pacific salmon (*Oncorhynchus* spp.) (e.g. Dorcey et al. 1978), particularly chinook (*O. tshawytscha*) which frequently spend more time in estuaries as juve-

niles than other salmon species (Healey 1982). With industrial and recreational development competing with salmonids for estuarine space, it is becoming increasingly important to understand the influence of estuarine residency on salmonid survival. Therefore, a study was initiated in whch juvenile chinook salmon smolts from the Quinsam hatchery, near Campbell River, B.C., were tagged, transported, and then released at four sites that differed in degree of marine influence. In this manner, some chinook were deprived of a period of estuarine residence. Returns of these fish to the hatchery and recoveries by sport and commercial fishermen are being monitored to determine if these is a relationship between estuarine residence and survival. Preliminary results of these returns are reported here, but final conclusions regarding survival await additional adult recoveries.

Observations made during and immediately following our releases of juvenile chinook salmon in the spring of 1983, 1984, and 1985 in four locations in the Campbell River region have allowed us to examine the following questions. (1) What types of predators and what level of predation are the released fish exposed to? (2) What effect does handling and transportation have on physiological stress levels and mortality rates and are stress levels related to the degree of marine influence at the locations in which the fish were released? (3) Does the diet composition, the time taken to initiate feeding on natural prey items, the amount of food consumed, or the growth differ among fish released at the four locations? (4) What patterns and rates of dispersal and residency times do fish released at each location show?

Materials and Methods

The Campbell River is located on the east coast of Vancouver Island, British Columbia (Fig. 1), draining a basin of 1741 km^2 into Discovery Passage. A salmon hatchery was constructed 6 km upstream of the river mouth on the Quinsam River. Chinook salmon are released from this facility each year.

During the spring of 1983, 1984, and 1985, approximately 140 000 chinook fry were reared in hatchery ponds in preparation for this experiment. Each fish was marked by adipose fin removal and a wire tag that identified release location was inserted into its nose cartilage. Fish were randomly divided into four release groups. Tagged fish were held in aluminum troughs (6.1 m × 81 m × 60 cm) for at least 2 d before being transported to the release sites. Further details on tagging procedure are presented in Levings et al. (1984) and Kotyk et al. (1985).

In late April of each year before the production releases of Quinsam Hatchery fish but during the period of peak outmigration of wild chinook fry (Levings et al. 1986), approximately 35 500 fish (2–4 g) were transported by helicopter to each of four sites. Before release, a subsample of fish from each release group passed a saltwater challenge test (Clarke and Blackburn 1977). Fish released in the marine zone were taken to Deepwater Bay (Fig. 1) (mean temperature 9.2°C, mean salinity 33.3‰, examined on the release date during the three years), while transition zone fish were released adjacent to the mouth of the Campbell River estuary (site 34, Fig. 1) (10.8°C, 11.7‰). Estuary zone fish were released at the mouth of Baikie's Slough (Station 7) (10.2°C, 2.9‰) and river zone fish were released into the Quinsam River adjacent to the hatchery (Fig. 1) (11.8°C, 0.0‰). All release groups of fish spent approximately 10 min in the air while being transported from the hatchery to the release sites.

A semi-collapsible rubber monsoon bucket, slung beneath a helicopter, was used to transport the fish to each site. The bucket received aeration from an attached oxygen cylinder. Several trips were made to each site to allow fish loading density to be kept well below the 0.12–0.25 kg fish/L water recommended by the Department of Fisheries and Oceans (1984) and FAO (Berka 1986). At each release site the bucket was lowered into the water (water depth 4 m) and doors on the bottom of the bucket were pneumatically opened to release the chinook.

Each year during the day of release, counts of potential avian and mammalian salmonid predators were made from the shore adjacent to each release site. At the marine release location, two SCUBA divers were in the water to observe the behaviour of the fish after release and to estimate mortality due to predators or release procedures. Divers observed the released fish from the surface for approximately 20 min before searching the bottom (3–14 m) for mortalities. Diver observations were performed only at the marine site because this is where we expected the largest mortality. Horizontal water visibility was in excess of 7 m throughout the water column.

On the first trip to each site, approximately 180 fish were removed by dip net and divided equally into three cages. The cages (80 × 50 × 60 cm, with 1-cm stretched mesh) were tied together and floated vertically at the surface in a location that protected fish from extreme wind and wave conditions and exposed the fish to temperature, salinity, and depth conditions similar to those of the release environment. The cages straddled the halocline at the estuarine and transition sites. They were examined once every 2 d throughout the 20-d experiment to count and remove dead salmon. Stomach contents of caged fish collected weekly (a total of 10 fish from each zone in 1983, 20 in 1984, and 22 in 1985) were weighed and identified in the laboratory.

In the four zones, immediately after delivery by helicopter, and for several days following delivery during each collection day, 10–12 fish were removed from cages (see Fig. 3 for collection days) to examine stress levels using three criteria. Plasma cortisol concentrations (acute response) were measured with a radioimmunoassay technique (Donaldson et al. 1984), interrenal cell nuclear diameters (ICND, sustained response) were measured histologically (Donaldson et al. 1984), and haematocrits (packed cell volume) were determined as a general health measure. Plasma cortisol concentrations in salmonids have been shown to respond to a wide range of major and minor stressors (Schreck 1981). Interrenal tissue, the structure that produces and releases cortisol, usually shows detectable responses only to major chronic stressors (Donaldson et al. 1984). Haematocrits may either increase or decrease in response to changes in environmental parameters, such as oxygen and temperature, or by stresses and diseases (Munkittrick and Leatherland 1983).

Stress estimates of fish held in the cages were compared with those made from fish collected from the hatchery holding troughs and stock ponds 1–2 d before the release. Collections from holding troughs and stock ponds were made in the morning prior to the commencement of operations to ensure that the fish were in an undisturbed state. Plasma cortisol, ICND, and haematocrit results were analysed separately for each year using two-way analysis of variance (ANOVA, factors are location and days spent in cages) and Tukey's multiple range tests (p = 0.05).

Each year, within a few hours of releasing the fish and then daily for a period of 4–5 d, replicate (n = 2–6) beach seines

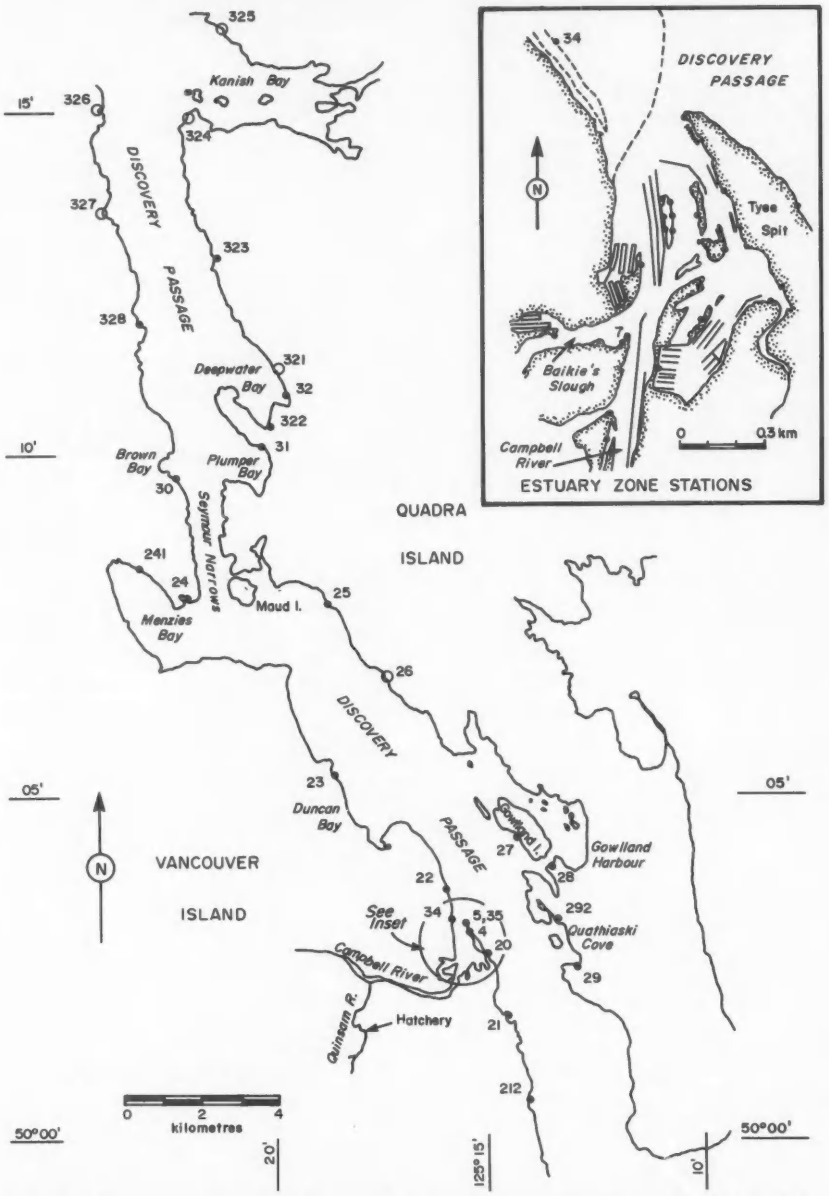

FIG. 1. Sampling area showing release sites in the Quinsam River (adjacent to hatchery), in the Campbell River estuary, in the marine zone adjacent to the mouth of the estuary (Stn. 34; transition zone), and at Deepwater Bay (Stn. 32; marine zone). Open and solid mark sites where beach seines were made during the first week after each year's release to recapture marked fish. Solid circles are sites that were seined biweekly for several months following the release.

were made at 45 sites in order to recapture marked fish (Fig. 1). Beach seines are the most appropriate method to recapture juvenile salmon because salmon remain in shallow nearshore regions during outmigration (Macdonald et al. 1987). Estimates of dispersion patterns, migration rates (distance travelled per unit of time), and feeding success were made for this period. We assumed that most chinook migration occurred northward (Ball et al. 1985; Waddell and Markowski 1983) and therefore sampled more intensively to the north of the estuary mouth. The 13.7-m seine was pulled from the beach using an inflatable boat with a 37-kW engine and was retrieved by hand. If sufficient numbers were caught, 10 tagged chinook salmon and members of all other species of fish that were suspected of preying on salmonids were collected at each site and fixed in 10% formalin solution. When more than 10 tagged salmon were captured, 10 fish were subsampled from the net. To estimate the number of

TABLE 1. Counts of potential juvenile salmonid predators and feeding observations made each year from the shore adjacent to each of the release sites during the 2–3 h that the releases were taking place (Fig. 1). Counts of dead fish observed immediately after the release are presented in the last column.

Site	Year	Predator	No. of predators	Feeding (+ yes; − no; ? undetermined)	Mortality
River	1983	None			None
(hatchery)	1984	None			None
	1985	None			None
Estuary	1983	Seal	1	?	None
(Stn. 7)		Herring gulls	20	+	
		Mergansers	5	+	
		Bald eagle	1	−	
	1984	Seal	1	?	None
		Mergansers	2	+	
	1985	Seals	2	?	35
		Bald eagles	2	−	
Transition	1983	None			
(Stn. 34)	1984	Bonaparte gull	40–100	−	
		Mergansers	4	−	
	1985	Seals	2	?	10
		Herring gulls	100	+	
Marine	1983	Bonaparte gulls	175–250	+	1
(Stn. 32)		Buffalo sculpins	12	+	
	1984	Bonaparte gulls	1000	+	None
		Dolly Varden	2	+	
		Buffalo sculpin	3	−	
	1985	Herring gulls	10	+	37

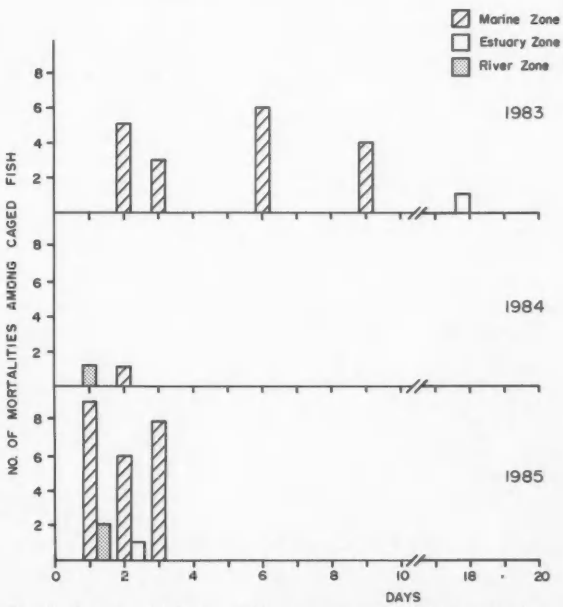

FIG. 2. Counts of daily mortalities among caged fish at each release zone, each year. No mortality was observed among fish kept at the transition zone.

fish of each release group caught in the seine the ratio of number of marked chinook from each release group in the subsample divided by subsample size (i.e. $n = 10$) was multiplied by the number of tagged fish in the entire beach seine catch. In the laboratory, fish were measured (fork length) and weighed, coded wire tags were removed and decoded, salmon stomach contents were weighed, and stomachs of potential salmonid predators were examined.

Between April and September (1983–85), nine additional beach seining trips were made biweekly to sample approximately 39 of the initial 45 sampling stations. Fish abundances and dispersal rates during this period were estimated. The same 39 stations were sampled repeatedly during the additional trips, and seining methods, subsampling methods, and number of seines remained constant.

Juvenile salmonid abundance and dispersal rates have usually been estimated using catch per beach seine (CPUE) data (e.g. Healey 1980). Because of the disparity in the sampling effort among zones (a much smaller percentage of marine habitat was sampled compared with the estuary), this method has a strong bias towards overestimating the number of estuarine- and river-released fish. Therefore, in this study the abundance and dispersion rate of each release group are estimated using only those sets that captured fish (i.e. zero catches were ignored). This approach to the problem assumes that juvenile salmon in estuaries and marine foreshore zones aggregate (Jaenicke et al. 1984) and uses, as an estimate of fish present, an estimate of the size of the aggregation.

Fish abundances of each of the four release groups during each of the three years were compared using a two-way analysis of covariance (ANCOVA, factors were year and release group) while controlling for and comparing the covariance due to declines in catch sizes among release groups with time (dispersion rates) (sequential sums of squares method, SAS Insti-

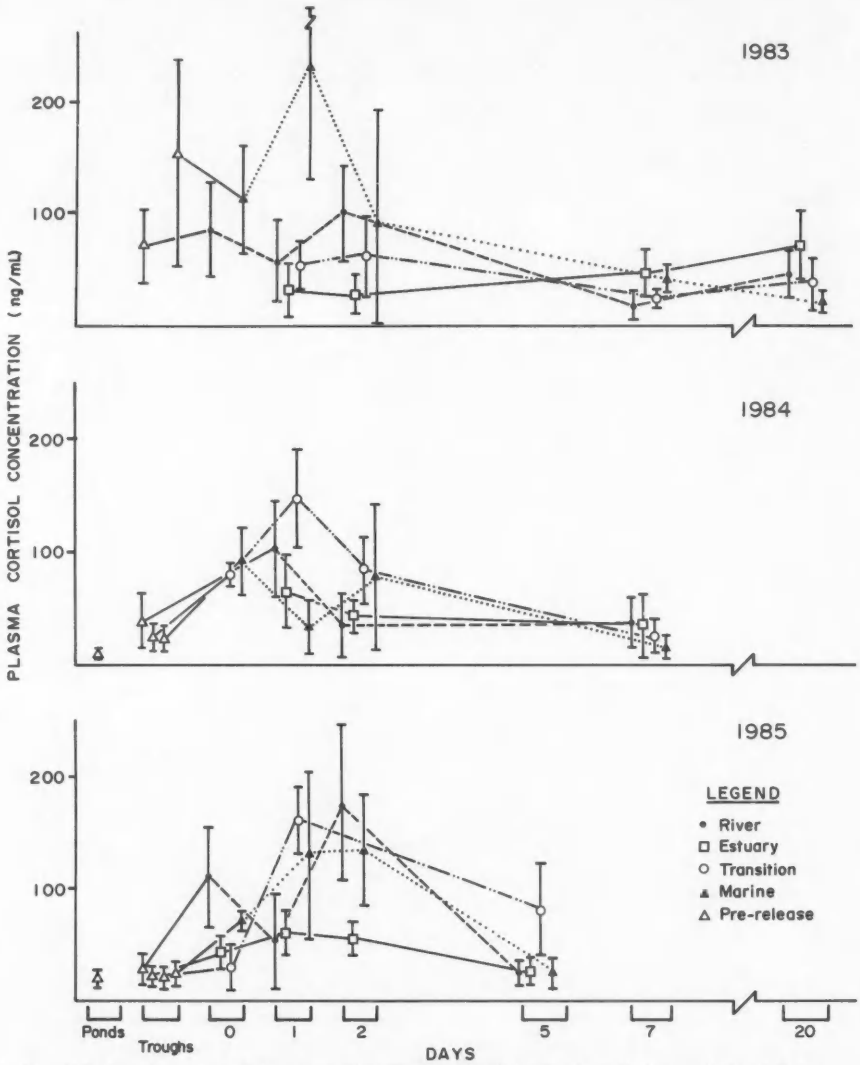

FIG. 3. Mean plasma cortisol concentrations in juvenile chinook salmon before and after helicopter transfer to cages at four sites during each year. Vertical bars depict 1 SD of the mean.

tute Inc. 1985). Using the mean catch size of each release group as input to the dispersion equation, estimates of residency times were made. Before analysis, data were transformed (ln + 1) to remove the dependence of the variance on the mean and to linearize the relationship between the dependent variable (catch size) and the covariate (trip number).

The ANCOVA procedure was also used to examine differences in stomach content weights of fish collected from cages among the release sites while controlling for and examining the covariance due to increased food intake with time. These data did not require transformation.

Growth rate estimates of fish from each release group were made by regressing fish weight on days since release. Regression coefficients were compared ($p = 0.05$) and 95% confidence limits were calculated.

Results

Release Observations

Predatory birds such as herring and Bonaparte gulls (*Larus argentatus* and *L. philadelphia*) and mergansers (*Mergus serrator*), present at the transition and marine release sites (sites 34 and 32, Fig. 1) each year during the day of the releases, were often seen feeding on juvenile salmonids (Table 1). Several Dolly Varden trout (*Salvelinus malma*) and buffalo sculpin (*Enophyrs bison*) caught by beach seine at Deepwater Bay has also consumed marked chinook salmon (2 and 11 chinook, respectively). Fewer avian or piscivorous predators were observed at the estuarine or river release sites (site 7 and the hatchery, Fig. 1). Most fish (e.g. flounders, surfperch, sculpins, salmonids) caught by beach seine in the estuary shortly

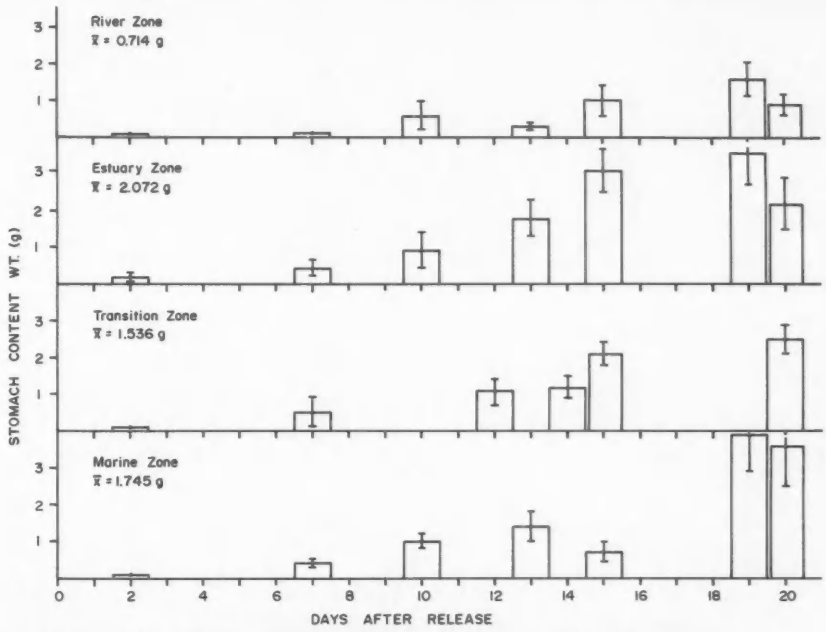

FIG. 4. Changes in stomach content weight with time after release for fish kept in cages in river, estuary, transition, and marine sites. Vertical bars describe 1 SE of the mean. Mean stomach content weights over the 20-d experiment are also presented.

after the releases were too small to prey upon the released fish. However, several bald eagles (*Haliaeetus leucocephalus*) and harbour seals (*Phoca vitulina richardi*) may have been attracted to the concentrations of hatchery chinook salmon in the estuary shortly after the release.

At all sites, each year, shortly after being released, fish were seen forming loose schools in shallow water near the shore. In 1985 at the marine site, this resulted in some fish (approximately 135) being stranded as the tide receded. Approximately 50 fish entered a small creek near the release site. Counts of dead fish by shore observers immediately after the release of fish from the helicopter were highest at the marine site (Table 1). A few additional mortalities observed by the divers and therefore not recorded on Table 1 were observed sinking into deep water at the marine site.

Fish were rarely seen more than 2 m below the surface by SCUBA divers at Deepwater Bay (site 32). During the initial 5 min after release, many fish were attracted to solid objects in the water such as pilings, rocks, and boats, and if they were not disturbed by sudden movement, fish congregated around divers in the water. Observations by SCUBA provided additional fish dispersion information. Immediately after the release the aggregations of fish began subdividing into looser and smaller assemblages. These fish appeared lethargic immediately after the release. Gradually, however, they began to respond to sudden movements made by divers, or wave action, by tightening their schools and rapidly escaping. Individual fish were seen making brief excursions into deep water (2 m), but very few were seen near the bottom when depths were greater than 2 m. Within 25 min of the release, fish were seen ingesting food (calanoid copepods) or nonfood items (drifting pieces of wood chips and seaweed).

Stress

Almost all mortality suffered by caged chinook occurred in the marine zone and occurred within 10 d of the initiation of the experiment (Fig. 2). Mortality at the marine site in 1985 was likely due to an accidental spill and subsequent handling of fish that were in the process of being transferred to the marine zone cages.

Haematocrit levels were not significantly influenced by experimental procedures in this study. However, measurements of plasma cortisol levels and ICND's indicated that there was some stress associated with the transfer of fish to each location, each year (Fig. 3 and 4; day effect, $p < 0.05$). Stress levels were highest during the first few days after the release and gradually declined to levels equal to or lower than those recorded before the transfer (Tukey's, $p < 0.05$). The degree of stress imposed by the transfer operation was dependent on the zone into which the fish were released (location effect and location × day interaction, $p < 0.05$). Higher stress levels were recorded among river- and marine- and in some cases transition-released fish than those recorded from the estuary. Higher initial stress levels (Fig. 3 and 5, troughs) in 1983 than in 1984 and 1985 may have been a response to the bright unpainted metal surface of the holding troughs. In later years the troughs were painted green to reduce reflected light levels.

Fish Feeding and Growth

Fish held in cages began feeding at all sites within 2 d of the start of the experiment (Fig. 4). During the time they were held in cages (approximately 20 d), stomach contents increased in weight ($p < 0.05$, Fig. 4). Rates of increase did not differ among sites but fish held in the river had the lowest average content weights over the 20-d trial (Duncan's multiple range test, $p < 0.05$).

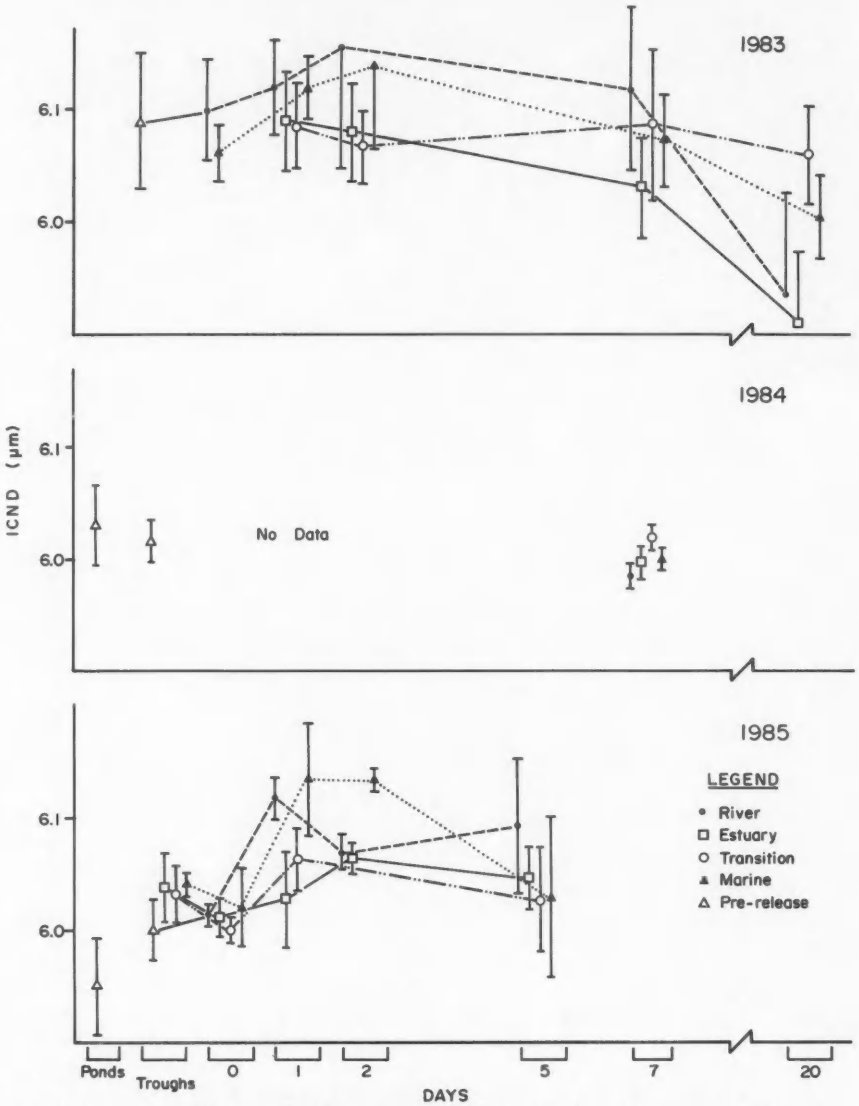

FIG. 5. Mean interrenal cell nuclear diameters of juvenile chinook salmon before and after helicopter transfer to cages at four sites during each year. Vertical bars depict 1 SD of the mean.

Fish of all groups caught by beach seine were eating natural prey within the first week after release. Content weights increased as the summer progressed and fish grew at a rate of approximately 1 g/wk (Fig. 6). The forage ratios (stomach content weight divided by body weight) ranged from 0.5–1.0 during the first week after release to 2.0–3.5 several months later (1983, $n = 79$; 1984, $n = 56$; 1985, $n = 88$). There were no consistent differences in amount of food eaten or growth rates ($p > 0.05$) among fish captured from each zone each year.

Consumption of food originating from marine habitats increased with increasing marine influence at the sites the fish were held in (Table 2). Fish held at the estuary or transition sites, in addition to consuming food of freshwater and estuary origin, consumed food of marine origin. Evidently fish were entering the lower, more saline portion of the water column within the cage to feed on marine plankton. Alternatively, marine plankton is mixed into the upper portion of the water column in the estuary.

Fish Dispersion

During the first week after each year's release, fish dispersed across Discovery Passage to both the north and south (Fig. 7). Some river- and transition-released fish travelled 10–20 km to regions north of Seymour Narrows. Marine-released fish travelled similar distances from Deepwater Bay. During the first week, marine-, river-, and transition-released fish, caught in the marine zone, travelled mean distances of 4.5, 6.0, and 7.5

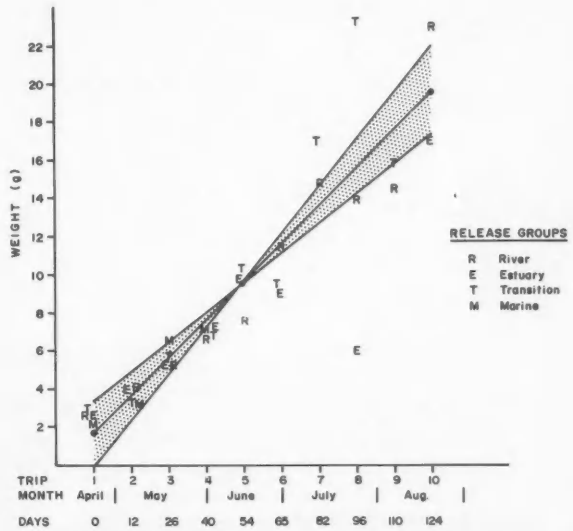

FIG. 6. Growth rate of chinook salmon (all release groups and all years combined) as described by a two-parameter model: weight = $-0.34 + 2.01 \times$ trip. Shaded region represents the 95% confidence interval about the growth rate estimate. A linear model best described the relationship. Mean weights of fish of each release group collected at each time are also presented (see legend).

km/wk, respectively. Estuary-released fish, however, were recaptured less frequently outside of the estuary than other release groups and were not recaptured at distances greater than 5 km from the estuary (mean dispersion rate = 0.5 km/wk) during the first week following release.

Each year, in the months following the experiments, similar numbers of each group of fish were recaptured (year effect, $p > 0.05$, Table 3; Fig. 8) and the dispersion rates of each fish group did not vary from year to year (year × release group effects, $p > 0.05$). Therefore, we combined data from all three years (Fig. 8). More estuary-released fish were captured than all other groups (release group, $p < 0.05$). Marine-released fish tended to disperse faster than fish released in other zones (Table 4; Fig. 8) despite the fact that a small number of them entered small creeks adjacent to the release site. River-, estuary-, and transition-released fish were captured up to 18–20 wk after release whereas marine-released fish had left within 8 wk of the release and few were captured in the estuary. Calculation of dispersion rate and residency time of fish released in the transition zone was complicated by the occurrence of increasing catch sizes during the first month after release, and therefore the dispersion rates for these fish were lower than expected (Table 4). For this reason an accurate residency time could not be calculated from the dispersion equation for these fish. The residency estimate presented in Table 4 for the transition release group was made by estimating the mean number of days between release and capture. An initial increase in catch sizes can be attributed to many transition-released fish returning to the estuary for a few weeks before passing back through the transition zone on their way seaward (vertical bars, Fig. 8). River-released fish showed a similar but smaller lag in peak abundance because they remained in the river for a period (approximately 1 mo) before entering the estuary.

Recoveries of experimental fish released in 1983 and 1984 and recovered up to the end of 1986 by the commercial or sport fisheries ($n = 163$) and escapements to the Quinsam River salmon hatchery and spawning grounds ($n = 24$) indicate that river- and estuary-released experimental chinook have survived better than those released in the marine or transition zones (63 recoveries from river, 69 from estuary, 35 from transition, and 20 from marine). All fish recovered at the Quinsam River hatchery in 1984 were precocious males. Due to the preliminary nature of these data, further interpretation must await additional recoveries.

Discussion

Chinook released in a manner that permitted association with the estuary showed many signs of delayed seaward migration. They dispersed at lower rates (Fig. 8; Table 4), travelled shorter distances immediately after release (Fig. 7), and thus were recaptured in greater numbers than chinook that were deprived of estuarine residence. Even fish released in the marine environment adjacent to the estuary (transition zone), which were apparently physiologically ready for marine conditions (i.e. seawater challenge test, Clarke and Blackburn 1977), showed

TABLE 2. Mean numbers of prey items eaten by chinook removed from cages during each year. Sample sizes of fish collected from each year are presented and prey items are categorized by origin.

Cage location	Year	n	Food type (no. of food items)			
			Freshwater	Estuarine	Marine	Nonfood items
River	1983	10	0.4	—	—	2
	1984	20	—	—	—	28
	1985	22	0.1	—	—	35
Estuary	1983	10	0.1	1.2	0.2	6
	1984	20	3.7	2.9	0.9	11
	1985	22	—	2.3	0.1	1
Transition	1983	10	1.3	—	5.7	—
	1984	20	9.2	0.5	18.4	40
	1985	22	—	0.5	3.0	12
Marine	1983	10	—	—	22.7	—
	1984	20	—	—	9.6	15
	1985	22	—	—	5.1	15

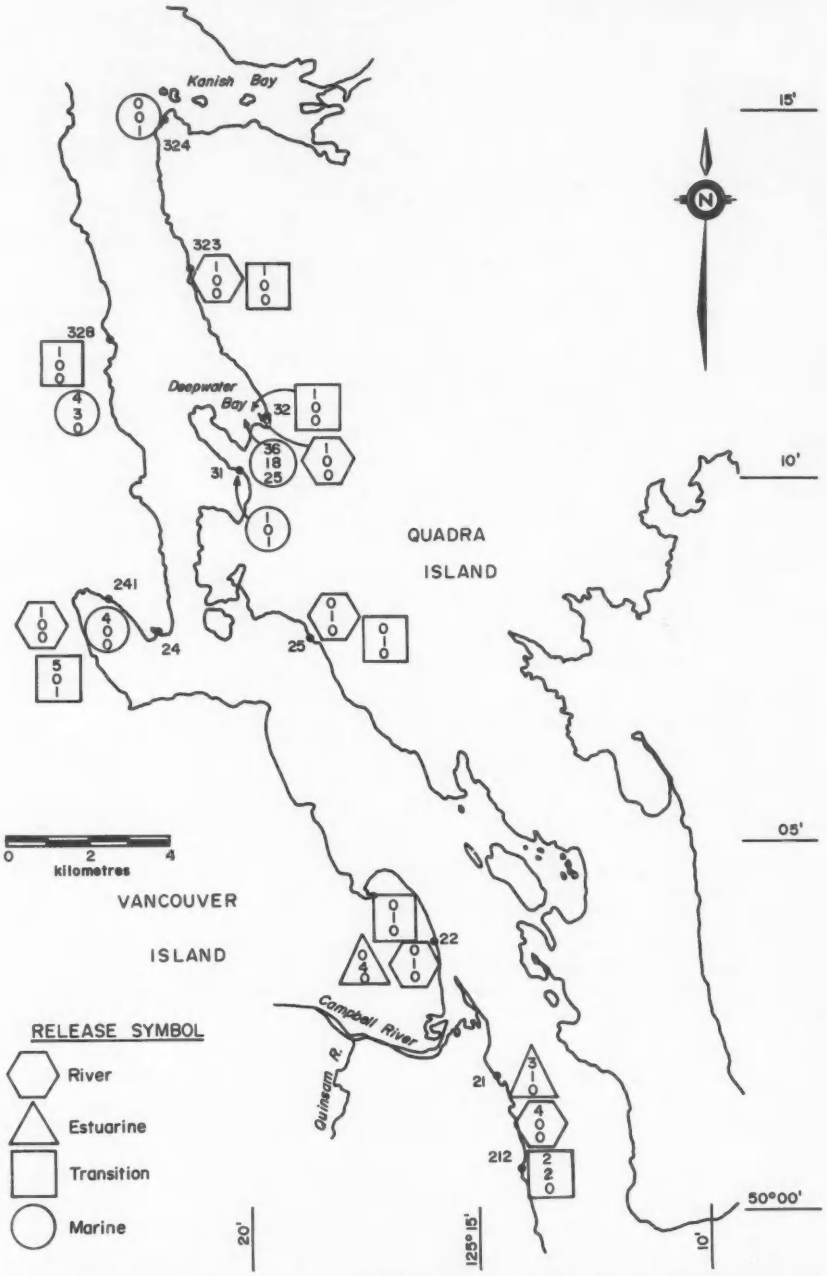

FIG. 7. Dispersion patterns of chinook from the four release sites (Fig. 1 and 2) during the first week after release. Within each symbol corresponding to a particular release group, from top to bottom, are given numbers of fish caught by beach seine during the first week after release in 1983, 1984, and 1985. Fish caught at sites within the estuary are not included.

a strong tendency to return to the estuary for a period before migrating seaward (Fig. 8). However, the rapid decline in numbers of marine-released fish recaptured may have been partially due to the increased levels of marine mortality (Fig. 2; Table 1).

Juvenile chinook are found in Pacific coast inshore regions for 3 or 4 mo during the spring (Healey 1982; Myers and Horton 1982; Levings et al. 1986). Healey (1980) estimated that chinook occupied the Nanaimo estuary and adjacent foreshore region for a mean of approximately 60 d. This was longer than the 21- to 47-d periods recorded for groups in this study (Table 4). Healey (1980) used a purse seine which allowed the capture of fish further from shore and therefore recorded fish

TABLE 3. ANCOVA table resulting from the analysis of changes in mean catch size among release groups and years with trip (Fig. 8). Analyses were performed using the SAS statistical package (SAS Institute Inc. 1985) sequential sum of squares approach, GLM procedure.

Source	df	SS	*F*	Probability
Year	2	1.10	0.61	0.54
Release group	3	8.65	3.19	0.02
Year × release group	6	8.80	1.62	0.14
Trip (covariate)	1	7.94	8.80	0.00

in the area for a longer period of time. Dispersal rates are also a function of the location where fish were released (Table 3, release group, $p < 0.05$). Residency times of fish released to their usual habitats (estuary and river location) indicated that chinook remained in the sampling region for periods approaching the 60-d estimate made by Healey (1980).

Inferential evidence suggests that extended estuarine use will increase chinook survival but little conclusive evidence exists. Until about 1990, monitoring of returns of our fish to the hatchery and recoveries by sport and commercial fisheries will continue and should provide a clearer understanding of this. Adult returns to date and short-term results presented here suggest that an early estuarine phase in the life cycle of chinook salmon can have a positive effect on survival, possibly due to benefits relating to predation, feeding, osmoregulation, and reduction in stress.

Estuaries may provide a sanctuary for salmon from some predators. Nonsalmonids that cohabit the estuary (sculpins and flounders) are generally smaller than those in the intertidal regions of the adjacent marine zone (McCabe et al. 1983). This was the case at the Campbell River estuary, also. While coho (*O. kisutch*), cutthroat (*Salmo clarki*), and Dolly Varden are cited as active salmonid predators in rivers (Pritchard 1936; Hunter 1959; Fresh et al. 1982), fish predation on salmonids in some estuaries is low (Myers 1978; McCabe et al. 1983). Recent stomach analyses of samples of 107 coho smolts captured in the Campbell River estuary in 1984 contained no salmonid remains (J. S. Macdonald, Department of Fisheries and Oceans, West Vancouver Laboratory, unpubl. data). Many fish found in the marine zone, including hake (*Meluccius productus*), rock fish (*Sebastes* sp.), mackerel (*Scomber japonicus*), and ling cod (*Ophiodon elongatus*), have frequently been recorded as salmonid predators (Anonymous 1959; Black and Low 1985; Ashton et al. 1985). Most avian predators also occurred less frequently at the river or estuary sites than at the more marine influenced sites (Table 1). However, current studies have shown that bird predation on salmonids in estuaries can be significant (Mace 1983; Wood 1987a, 1987b). Examination of daily and seasonal changes in abundance of both avian and fish predators is required before we can fully appreciate the importance of estuaries as refuge areas for salmonids.

Extended residence in estuaries, including temporary return to the estuary following the release in the sea (e.g. our transition zone released fish), may be related to the availability of suitable food resources in the estuary (Mason 1974). The marine food that enters the estuary via the tidally influenced saltwedge (Macdonald et al. 1987) in conjunction with river and estuary food sources may provide large varieties and amounts of potential food items. In the estuary and transition zones, diet composition of chinook and their stomach content weights may reflect the diversity of available food in the immediate environment (Kask et al. 1986) (Table 2; Fig. 4). Salmon in river

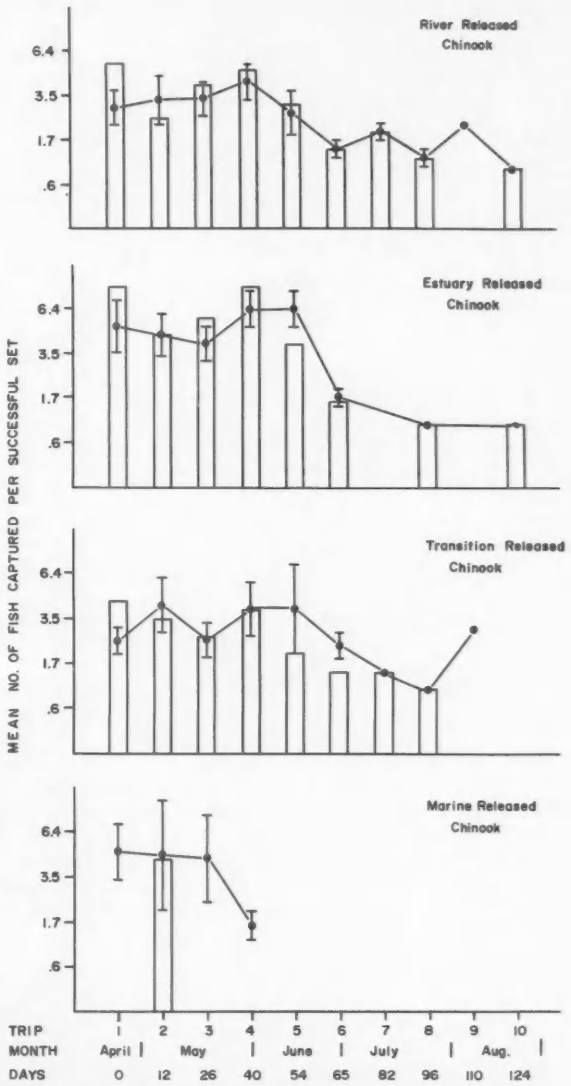
River Released Chinook
Estuary Released Chinook
Transition Released Chinook
Marine Released Chinook
MEAN NO. OF FISH CAPTURED PER SUCCESSFUL SET
6.4
3.5
1.7
.6
TRIP 1 2 3 4 5 6 7 8 9 10
MONTH April May June July Aug.
DAYS 0 12 26 40 54 65 82 96 110 124

FIG. 8. Descriptions of the dispersion pattern of each release group using fish sampled from all sites (Fig. 1) from April to August. Data are mean numbers of fish caught (±1 SE of the mean) during each successful seine (i.e. sets in which no fish were caught are not included). The results of a ANCOVA performed on these data are reported in the text. Vertical bars represent mean numbers of fish from each release group that were caught in the estuary.

habitats often have less food in their stomachs than those in estuaries (Congleton et al. 1981) (Fig. 4).

There are indications that fish deprived of an estuarine residency period will suffer higher degrees of stress than those released into estuaries (Fig. 3 and 5). While most fish in this experiment experienced a transient stress response after the transport procedure, those released into the marine environment may have had difficulty adjusting to the sudden increase in salinity. The stress of physiologically adapting to salt water may have added to the stress levels already imposed by transportation and confinement to cause higher mortality in marine-

TABLE 4. Estimates of dispersion rates from the Campbell River estuary – Discovery Passage area of each release group of fish. Rates are the slope coefficients that result from regression of data presented in Fig. 8. Estimates of mean residency time in the Campbell River region are also presented. They are calculated from the dispersal rate regression equations except for transition-released fish where mean number of days between release and capture was used as an estimate of residency time (asterisk).

Release group	Rate	Mean residency time (d)
River	−0.070	47.3
Estuary	−0.100	33.8
Transition	−0.002	23.2*
Marine	−0.160	20.7

released fish than others. Problems associated with scale loss have been shown to be more acute among fish released into marine water than freshwater (Bouck and Smith 1979). However, transient high stress levels among fish released to other zones suggest that additional factors may have an effect. Differences in currents, temperatures, and noise levels (boat traffic, river rapids) were beyond our control and may have imposed differential levels of stress among zones. Interestingly, interrenal cell nuclear diameters in 1983 were initially slightly larger than in the following years and were still decreasing after day 7. In 1983 the smoltification process may not have been complete at the time of transfer. Interrenal activity in salmonids is known to show transitory increases prior to smoltification (Olivereau 1962; McLeay 1975; Specker and Schreck 1982).

The results of this study support the hypothesis that it is advantageous for juvenile chinook salmon to remain in estuaries for an extended period. Without an estuarine residency period, or if chinook are forced through an estuary prematurally due to estuarine degradation or overcrowding, juvenile survival will likely be reduced. Further understanding of the importance of estuaries to salmonids awaits the return of our fish to the hatchery or recoveries by commercial and sports fisheries.

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Oxygen Isotope Studies on Sea Scallops, *Placopecten magellanicus*, from Browns Bank, Nova Scotia

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Oxygen isotope records from two sea scallops, *Placopecten magellanicus*, collected alive from Browns Bank, Nova Scotia, show annual cycles that closely approximate the isotopic composition predicted as a function of observed temperatures and the isotopic composition of the ambient seawater. The external growth lines coincided with the most positive $\delta^{18}O$ values of the cycles, suggesting their formation during the period of coldest water temperatures (spring). The $\delta^{18}O$ results indicate that the growth lines are annual events, consistent with the biological evidence.

Les données sur la présence de l'isotope d'oxygène dans deux pétoncles géants, *Placopecten magellanicus*, capturés vivants sur le banc de Brown (Nouvelle-Écosse), montrent des cycles annuels qui se rapprochent de la composition isotopique prédite comme une fonction des températures observées et de la composition isotopique de l'eau de mer ambiante. Les courbes de croissance externe coïncidaient avec les valeurs $\delta^{18}O$ les plus positives des cycles, ce qui donne à penser que leur formation a lieu au cours de la période où les températures de l'eau sont les plus froides (printemps). Les résultats de $\delta^{18}O$ indiquent que les courbes de croissance sont des événements annuels, qui sont conformes aux indications biologiques.

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The sea scallop, *Placopecten magellanicus*, is an important economic resource for Atlantic Canadian fisheries, providing the second highest income from landings. Sea scallops in Atlantic Canada inhabit water depths from approximately 1 m below mean low tide to deeper than 100 m (Culliney 1974; Mohn et al. 1985). The considerable economic importance and heavy exploitation of sea scallop stocks underscore the need for accurate age and growth rate estimates for this species. Such information is vital if the sea scallop fishery is to be managed effectively.

The techniques that are presently employed for age determination of the sea scallop rely on the interpretation of lines visible on the exterior of the shell or on the hinge ligament (Stevenson and Dickie 1954; Merrill et al. 1965). The external lines are assumed to be deposited in early spring each year. Although the technique is used extensively by both the Department of Fisheries and Oceans in Canada and the National Marine Fisheries Service in the United States, it can present problems. Shock marks, due to a sudden change in temperature or injury by fishing gear, may be confused for annuli. Recognition of an annual ring is, at times, very subjective. The first annual ring may not be laid down during the first winter, but only in the second winter (Merrill et al. 1965). In view of the uncertainties involved in the accurate interpretation of annuli, additional independent methods of establishing the periodicity of these features would be useful. Furthermore, verification of annual formation would have significant management implications, since the exploitation rates are high enough that scallops tend to be captured in rather early growth phases.

The stable oxygen isotope method has recently been used successfully in the interpretation of annual growth patterns in bivalve shells (Jones et al. 1983; Margosian et al. 1987). This method has also been used on two specimens of sea scallops from the coast of Virginia by Krantz et al. (1984). Growth rates determined from the isotopic records by Krantz et al. (1984) are roughly twice those estimated from the external line method. No explanations were offered for this discrepancy. Furthermore, Krantz et al. (1984) reported that the external lines, or rings, which are presumed to be deposited annually in the spring, appear to occur in various seasons, according to the inferred seasonality of the isotopic profile.

The purpose of this study is to compare the age and timing of formation of external rings of sea scallops from Browns Bank, as determined by examination of the external lines and the oxygen isotope method. This should also help resolve the discrepancies reported by Krantz et al. (1984).

Materials and Methods

Living specimens of sea scallops used in this study were collected from Browns Bank, Nova Scotia. This location was cho-

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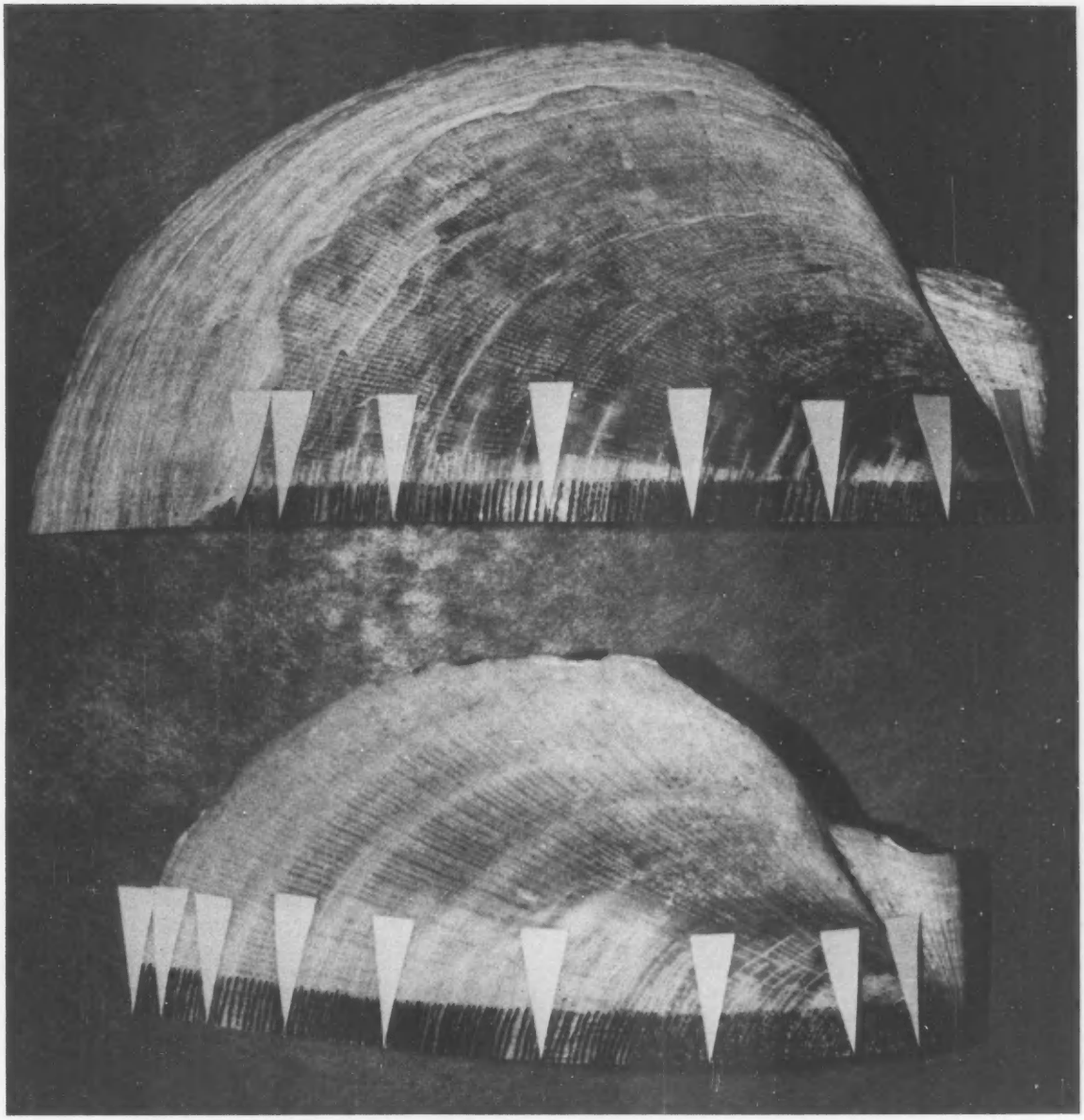

FIG. 1. Shells 1 (top) and 2 (bottom) showing sampling lines (marked in black for visibility) and growth lines (arrowheads).

TABLE 1. Monthly mean water temperature, salinity at C3, and predicted $\delta^{18}O$ for shell calcite.

Date	Temp. (°C)	Salinity	$\delta^{18}O_{water}$	$\delta^{18}O_{shell}$ (predicted)
April 1979	4.64	32.74	−1.24	2.00
May	5.43	32.87	−1.18	1.83
June	6.31	32.89	−1.17	1.59
July	7.69	33.35	−0.93	1.44
August	9.43	33.64	−0.77	1.12
September	10.94	33.99	−0.59	0.90
October	11.33	34.13	−0.52	0.87
November	11.63	34.07	−0.55	0.76
December	10.22	33.80	−0.69	0.99
January 1980	6.88	33.15	−1.03	1.57
February	5.25	32.77	−1.23	1.84
March	4.60	32.79	−1.22	2.04
April	4.76	33.11	−1.05	2.16
May	5.34	33.07	−1.07	1.96
June	6.50	33.17	−1.02	1.68
July	7.31	33.22	−0.99	1.48
August	8.57	33.43	−0.89	1.24

$^{a}\delta^{18}O_{water} = 0.523 \times \text{salinity} - 18.179$.

sen because there existed both commercial quantities of scallops and a long-term record of bottom temperature and salinity.

Isotopic analyses were performed on two specimens collected alive on May 20, 1983, and August 27, 1985 (42°53′20″N, 65°54′50″W at a water depth of 100 m for specimen 1 and 42°50′32.9″N, 66°07′31.2″W at a water depth of 85 m for specimen 2). The exterior of both shells showed no evidence of boring or extensive erosion; however, the outer shell margin of specimen 2 was unreadable, and so the exact age cannot be accurately determined. It does serve the purpose of comparing with the isotopic record from specimen 1.

Estimation of the yearly growth for the left (upper) valve of both shells was made using lines on the exterior of the shell following the method described by Merrill et al. (1965). Growth lines, representing estimated years of growth, were marked on each of the two shells by two experienced readers, D and M, of the Department of Fisheries and Oceans, Halifax, Nova Scotia, and were used for comparison with the growth interpretation based on the oxygen isotope method. The two shells used in this study were aged twice by both readers on two separate occasions and without reference to the results from the oxygen isotope analysis or other readings.

Reader D had aged shell 1 when it was first collected for another study in 1983 and again in 1986 as part of this study, but before shell samples were obtained for isotopic analysis. Shell 2 was first aged by this reader in 1986, before shell samples were obtained, and again in 1987, after sampling.

Reader M was also given the shells to age as a comparison between readers. The first occasion was before isotopic sampling in 1986 and the second time was in 1987, after sampling had taken place. On the first occasion, reader M was given the shells while he was in the process of ageing scallop samples from Georges Bank. He was told the two shells were from Browns Bank, an area with a slower growth rate than Georges Bank (Robert et al. 1985), and was simply asked to mark on the shells what he would consider to be annual rings. The second time he was asked to carefully study the shells and determine the exact position of the annual rings.

The two shells were prepared for isotopic analysis by first lightly sanding the exterior of the shell to remove the periostracum and any foreign matter. Discrete samples of shell material were then ground from the outer shell layer using a 0.5-mm Dremel bit. The calcium carbonate powders were collected approximately every 1 mm along the axis of maximum growth from about 10 mm from the umbo to the ventral margin (Fig. 1). Samples were taken only from the outer shell layer which is deposited sequentially along the shell margin during growth of the scallop. This was later verified using thin sections of the shells taken through the sampling lines. Attempts to sample close to the umbo were not successful, as the argonitic pallial myostracum layer was too near the surface to get a discrete sample from the outer shell layer that was large enough for analysis.

The stable isotopic composition of each shell powder sample was determined using a modification of techniques described in Tan and Hudson (1974). Approximately 1 mg of sample was placed in a pyrex thimble and roasted in vacuo at 400°C for 1 h. Once cool, roasted samples were reacted in vacuo with 100% phosphoric acid at 25°C for 12 h. The oxygen isotopic composition of the evolved CO_2 gas was determined using a 60° sector isotopic ratio mass spectrometer, equipped with a triple collector and an on-line data processing calculator. The isotopic values are reported in the conventional delta (δ) notation as the enrichment or depletion of ^{18}O (parts per thousand, ‰) relative to the Peedee belemnite (PDB) carbonate standard (Epstein et al. 1953). The precision of the isotopic determinations was evaluated by analyzing an internal inorganic carbonate standard with every batch of 20 samples. The overall single standard deviation was ±0.11‰ (n = 39).

The oxygen isotopic composition of the seawater samples, used to determine the composition of the bottom water, was determined by the method described by Tan and Strain (1980) with a precision of ±0.1‰ (1 SD).

Hydrographic Conditions and the Predicted Isotopic Composition of Shell Calcite

Average monthly temperature and salinity data for April 1979 to August 1980 were obtained from station C3 (42°51′24″N, 65°49′30″W), 8 m from the bottom near the study site on Browns Bank (Table 1). The highest salinity (34.1‰) occurred in fall, while the lowest value (32.7‰) occurred in spring. The monthly temperatures show similar changes, with the highest temperature (11.6°C) occurring in November and the lowest (4.6°C) in March. Detailed temperature and salinity records for other years are not available for the study site, but data from a nearby station, C2 (43°03′18″N, 65°45′18″W), are compared in Fig. 2a and 2b, up to May 1983 when shell 1 was collected. The C2 record follows the pattern shown at station C3 in 1979–80. Temperature and salinity values for the near-bottom water in the surrounding area (42°48.20′ to 42°58.20′N, 65°49.50′ to 65°59.50′W) during 1920–80 also have similar ranges and variations as in 1979-80 (Department of Fisheries and Oceans, Marine Environmental Data Service, Ottawa, Ont., unpubl. data).

The oxygen isotopic composition of the bottom water at the study site was obtained by direct measurement of the near-bottom water in May and August 1985 and by measurement of $\delta^{18}O$ and salinity of bottom water in the nearby area (41°44.36″ to 43°03.72′N, 65°36.82′ to 67°20.10′W) during October 1986.

The expected isotopic composition of the shell calcite can be calculated by using (1) the paleotemperature equation of Epstein et al. (1953) and later modified by Craig (1965), (2) the mean

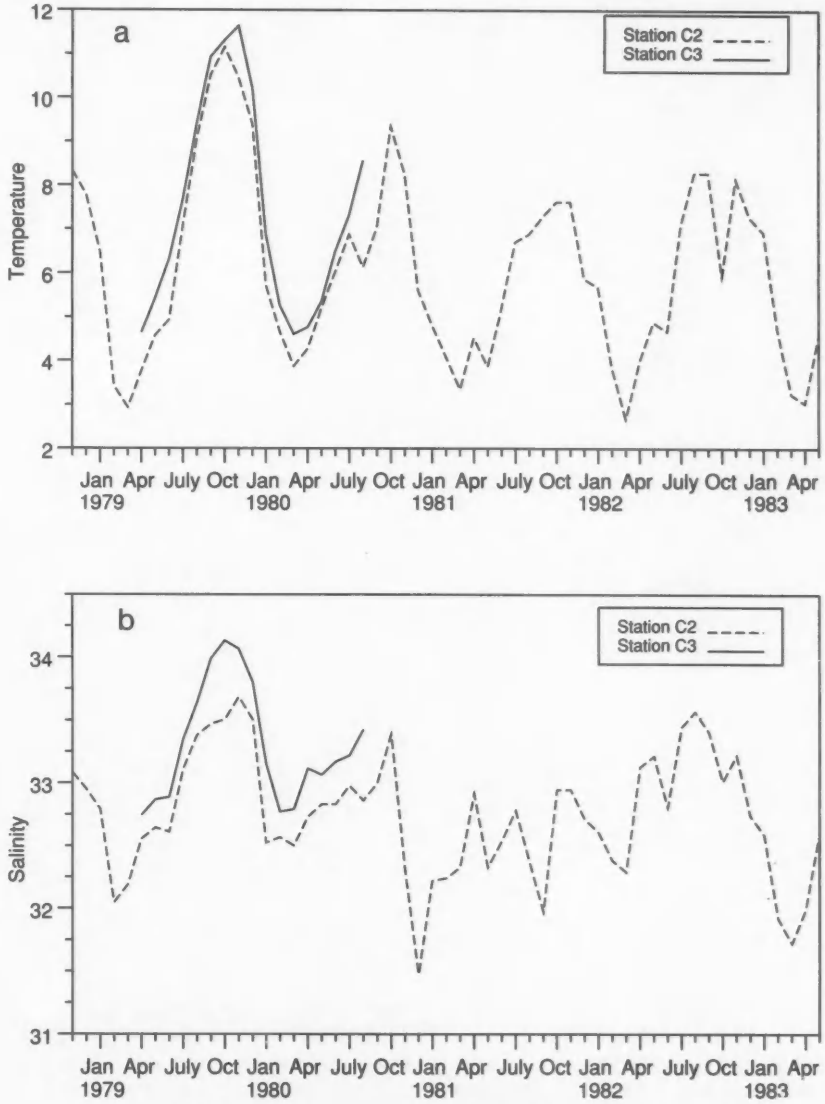

FIG. 2. Monthly mean (a) temperatures and (b) salinities at stations C2 and C3.

monthly bottom water salinity and temperature observed during April 1979 to August 1980, and (3) the oxygen isotopic composition of the bottom water at the study site. The predicted oxygen isotopic composition of calcite can be estimated by solving the modified paleotemperature equation of Craig (1965) using the quadratic formula, such that

$$\delta^{18}O_{(calcite)} = \delta^{18}O_{(water)} + \frac{[4.2-\sqrt{17.64-0.52(16.9-T)}\,]}{0.26}$$

where T = ambient temperature (degrees Celsius).

The oxygen isotopic composition and temperature of the water were substituted into the equation to obtain the average oxygen isotopic composition of shell calcite deposited during each month from April 1979 to August 1980 (C3) and November 1978 to May 1983 (C2).

Since the expected isotopic composition of shell calcite is determined by the temperature and the oxygen isotopic composition of the ambient water, it is essential to know the relative importance of these two environmental parameters. To this end we have used a constant $\delta^{18}O$ value of $-0.95‰$ (mean $\delta^{18}O$ values of water during 1979–80) and calculated the expected isotopic composition of shell calcite. The expected $\delta^{18}O$ curve is plotted in Fig. 3b.

Results and Discussion

The first reading by reader M (shell 1, 1986) differs from the other three readings (Table 2) and shows the high degree of subjectivity involved in the ageing process. Expecting to find rings closer together on shell 2 than on those from Georges

TABLE 2. Results from ageing of shells by reading external lines by two readers at different times. — = unable to determine exact position of ring.

Ring (age)	Shell 1: distance from umbo (mm)			
	D		M	
	1983	1986	1986	1987
1	—	10.0	—	—
2	—	21.2	—	—
3	47	40.7	32.6	52
4	66	63.2	54.2	68
5	81	81.6	69.3	82
6	95	95.0	82.7	93
7	106	106.2	95.4	103
8	113	113.6	105.9	108
9	118	118.5	113.9	113
10			118.9	—[a]

Ring (age)	Shell 2: distance from umbo (mm)			
	D		M	
	1986	1987	1986	1987
1	9.0	10.0	—	—
2	23.7	24.4	—	—
3	40.2	40.7	27.0	48
4	57.9	59.6	43.5	63
5	76.4	76.2	56.3	80
6	95.3	95.8	65.0	92
7	111.0	111.8	74.0	104
8	116.4	116.5	81.5	116
9			88.9	
10			94.9	
11			103.9	
12			110.6	
13			115.4	

[a]Edge of shell removed for sample prior to ageing.

Bank that he had just been ageing, that is what he found. The more careful examination when doing the second reading does indicate that close examination yields greater consistency between different readers.

There is a high degree of consistency in the two readings by reader D (Table 2). With the possible exception of ring 3 on shell 1, the positions assigned are within the bands of slow growth. There is also good agreement between D's readings and those from M's second reading, the major exception being that in shell 1, instead of a single ring at approximately 106 mm, M has said that there are two rings, one at 103 mm and another at 108 mm. As well, the positions of ring 3 in shell 1 and rings 3 and 7 on shell 2 differ between the two readers. The edge of shell 1 had been ground off for a sample before M's second reading, accounting for the lack of a ring around 118 mm.

Some differences in the position of the rings are expected when using external rings. The bands of slow growth are often several millimetres wide, and so differences in assigning a position to the exact point of slowest growth are expected. The presence of shock marks also has a large influence. When ageing a large number of shells, those that have an abundance of shock marks are often discarded as unreadable, or are left until the examination of the more readable shells has given the reader a "feel" for where the annual rings are expected to occur.

Differences in determining the total number of rings, and thus the age of the shell, are not as common, however, and so the difference between the two readers in assigning one versus two rings to the 100- to 110-mm area of shell 1 is more disturbing, and was unexpected.

The relationship between $\delta^{18}O$ and salinity as determined from the water samples was

$$\delta^{18}O = 0.523 \times \text{salinity} - 18.179,\ n = 19,\ r^2 = 0.98.$$

The monthly mean temperatures, salinities, and predicted $\delta^{18}O$ for station C3 are presented in Table 1 and both stations are compared in Fig. 3a. The predicted annual isotopic record from scallop shell calcite has a distinct periodicity and a $\delta^{18}O$ range of approximately 1.40‰. For station C3 the highest value (2.16‰) occurs in April 1980 and the lowest value (0.76‰) in November 1979 (Fig. 3b). Since the curve is based on the hydrographic conditions of April 1979 to August 1980, any other years of temperature and oxygen isotopic composition of bottom water may deviate somewhat from this predicted curve and would be expected to show up in the shell isotopic record. This can be seen in the year-to-year variation exhibited by station C2 in Fig. 3a.

The results of using a constant $\delta^{18}O$ value (Fig. 3b) show that the constant $\delta^{18}O$ water curve closely parallels the curve constructed with variable monthly temperature and $\delta^{18}O$ records, but the isotopic amplitude is larger by about 0.6‰. This shows that temperature is the dominant factor in determining the isotopic composition of shell calcite and that the variation of $\delta^{18}O$ of ambient waters has only relatively minor effect.

The $\delta^{18}O$ results for specimen 1 (Fig. 4) were fitted so that the April values correspond to the D-1986 ring positions, with the rest of the points linearly fit, with no attempt to compensate for seasonal growth differences. This specimen, with a shell height of 120 mm, was aged by the two readers, D and M, to be 9 and 10 yr old, respectively.

The oxygen isotopic record from specimen 1 exhibits several cycles; the most positive $\delta^{18}O$ value recorded is 1.9‰ and the least positive is 0.1‰. The isotopic amplitude of cycles vary from approximately 1.0 to 1.4‰. The annual growth lines appear to coincide with the most positive $\delta^{18}O$ values of the shell.

The $\delta^{18}O$ profile for specimen 2 also shows several cycles, with the isotopic amplitude varying from 0.9 to 1.3‰ (Fig. 5), very similar to specimen 1. The $\delta^{18}O$ values for the whole shell vary from 0.4 to 1.8‰, again very similar to the range observed for specimen 1. The annual growth rings also coincide with the most positive $\delta^{18}O$ values of the shell.

Based on the model described previously, the oxygen isotope records obtained from the scallop shells may be interpreted as yearly cycles controlled by seasonal changes in temperature and isotopic composition of the ambient waters.

Differences between the predicted and observed $\delta^{18}O$ values for shell 1 could be caused by several factors, one of which is a declining growth rate. As the growth rate of the scallop declines with age, the amount of shell deposited per unit of time also declines. This means that samples of shell that are of the same width and depth represent a longer period of time when taken from the ventral, slow-growing portion of the shell than the more dorsal, fast-growing region. This would have the effect of averaging the results from the outer region over a greater period of time and thus result in a reduction of the isotopic amplitudes of the cycles in this region. This sampling effect may account for differences in the amplitudes among the cycles, especially those in the region from 80 to 120 mm, and between the observed and predicted $\delta^{18}O$ values. The difference in the height of the cycles may be a result of using monthly

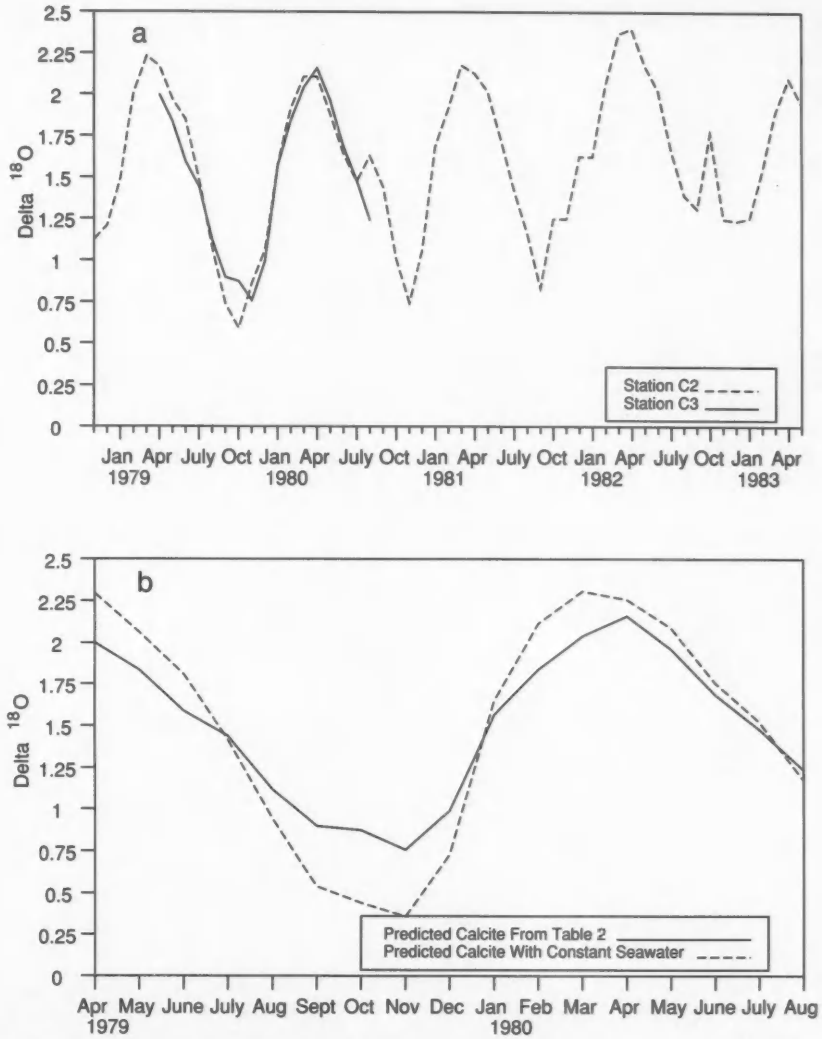

FIG. 3. Predicted isotopic values of shell calcite at (a) stations C2 and C3 and (b) station C3 with and without a constant $\delta^{18}O$ seawater value.

mean values of temperature and salinity. Broom and Mason (1978) showed that for *Chlamys opercularis*, although a shell lamella is probably laid down in 1 d, a lamella is not necessarily laid down every day. They attribute a cessation of shell growth to low temperatures and food availability. Thus if growth is only taking place during the warmer days in the month, there would be a shift towards warmer $\delta^{18}O$ values from that predicted with monthly mean temperatures. Another possible factor that may contribute to the differences in isotopic amplitudes is that there may be a slight difference in ambient temperatures and the isotopic composition of the water between the C3 and C2 sites and the actual point of capture of the scallops. Further studies would be required to resolve this.

There is a lot of within-cycle variability in the $\delta^{18}O$ signal as well. This may be explained by the within-month variation in temperature at these sites. Although the monthly mean temperatures give a smooth cycle, there is considerable within-month variation in temperatures in the Browns Bank region. At the C2 site for the study period (November 1978 to May 1983) the standard deviation of temperature within each month (usually sampled every half hour) averaged 0.81°C with a range of 0.27°C for July 1981 to 2.66°C for November 1980. Thus even if calcite is only being deposited in the warmer periods within a month a large variation in the $\delta^{18}O$ signal would remain.

Ambient temperatures during shell formation may be estimated from the calcite paleotemperature equation of Craig (1965) using the $\delta^{18}O$ values of the individual carbonate samples and an average water $\delta^{18}O$ value of $-0.95‰$. The estimated range of temperatures during shell deposition is 5.6–12.6°C for shell 1. This is very close to the minimum (4.6°C) and maximum (11.6°C) temperatures observed during April 1979 to August 1980 (Table 2). The good agreement between the isotopic and observed temperatures suggests that the shell carbonate was deposited in isotopic equilibrium with the ambient water.

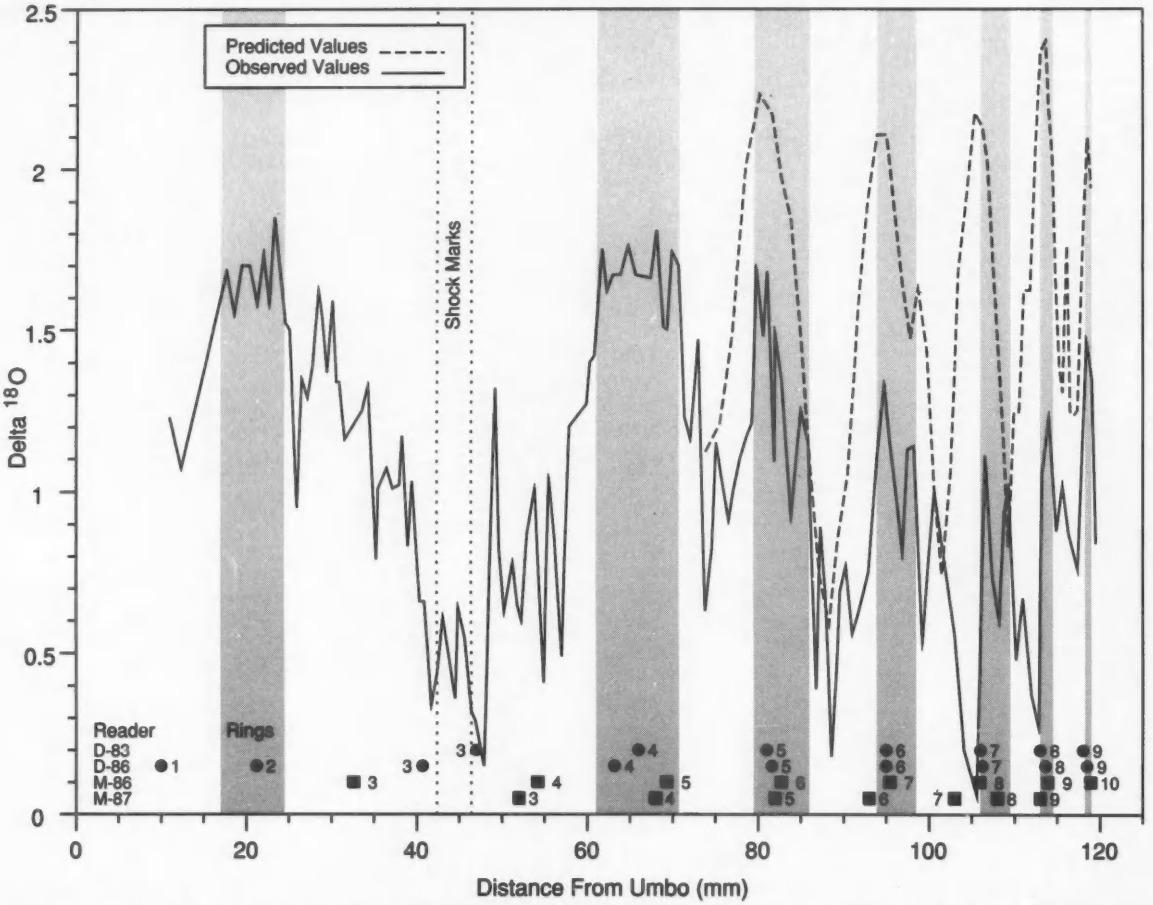

FIG. 4. Observed and predicted isotopic values for shell 1 along with the ring positions assigned by the readers as in Table 1. The shaded areas are subjectively assigned to observed isotopic peaks (cold water temperatures).

The $\delta^{18}O$ results for shell 1 indicate a good agreement between the 9 yr of growth interpreted from the external lines and the $\delta^{18}O$ record. Furthermore, the external lines, with the exception of ring 3, which are presumed to have formed annually in the spring, coincide with the most positive $\delta^{18}O$ values of the isotopic cycles. This suggests that the external lines are annual events and that they were formed during the coldest part (spring) of the year. The area of shell 1 in which the readers have assigned a ring number 3 is problematic. Biological literature indicates that it represents too large an increment to be considered a single year's growth. One explanation may be offered by the presence of two small shock marks at 42.4 and 46.4 mm. If shell growth ceased during these periods the peak in the cycle may not have been recorded in the shell. This would also explain the great variation in assigning a ring position to this area. Both readers felt that there should be a ring and yet are not consistent in where they place it. The reason could be that the slow growth band is missing. In shell 2, however, there is a similar section in which both readers have assigned ring 4. There is no evidence of shock marks in this area, and assigning one isotopic cycle from 35 to 60 mm and another from 60 to 85 mm would not agree with what is known about the growth rates for this area (Robert et al. 1985). This is either an extremely fast-growing individual and both readers are wrong in assigning a ring to this area, or for some environmental or physiological reason, there is a $\delta^{18}O$ cycle not recorded in the shell. More work would be required to resolve this.

Although the outer edge of specimen 2 is unreadable, its isotopic record does provide a good comparison with the record of specimen 1. Specimen 2 also shows several isotopic cycles from the umbo to a shell height of 120 mm. The $\delta^{18}O$ range of the shell (0.4 to 1.8‰) and the amplitudes of the cycles are very similar to those of specimen 1. The $\delta^{18}O$ range is also similar to the predicted $\delta^{18}O$ range of shell calcite. The locations of the rings again coincide with the most positive $\delta^{18}O$ values of the cycles.

Our results are not consistent with those of Krantz et al. (1984) who reported that the external lines are not annual events and that they were formed at different seasons of the year. Although the position of the rings in their sample agrees with that found by Posgay and Merrill (1979) for samples from the same area, their isotopic profile does not agree with the rings. In the two shells sampled in their study, some of the rings appear to coincide with seasonal maximum temperatures. There does appear to be a ring deposited during periods of minimum tem-

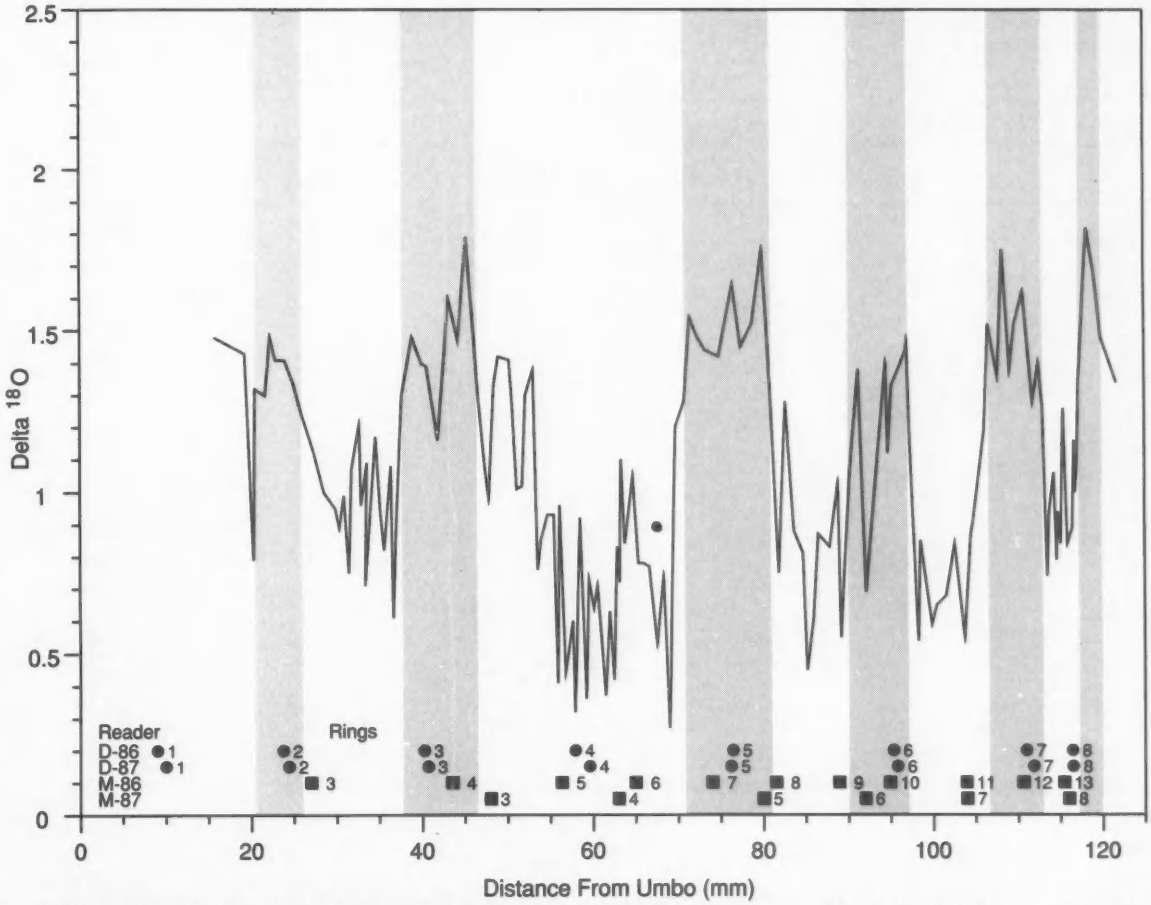

FIG. 5. Observed isotopic values for shell 2 along with the ring positions assigned by the readers as in Table 1. The shaded areas correspond to isotopic peaks (cold water temperatures).

peratures as well, but only after the second year of growth, i.e. rings 1, 3, and 5 in their specimen PM26 and ring 1 in PM10.

The reason for the difference in the results of the two studies may, therefore, be due to the temperature regimes of the study sites. The warmer, more southern site may produce a summer growth check during maximum water temperatures, while allowing for good growth in winter during the early, fast-growing years; the more northerly Browns Bank site may have the single growth check during the period of minimum water temperatures as has usually been reported in the literature.

The oxygen isotopic records from two scallop shells collected alive from Browns Bank show annual cycles that closely approximate the isotopic composition predicted as a function of observed temperatures and isotopic composition of the water. The external growth lines appear to coincide with the most positive $\delta^{18}O$ values of the cycles, suggesting their formation during the period of coldest water temperatures (spring). The $\delta^{18}O$ results indicate that the growth lines are annual events, consistent with the biological evidence. Thus the $\delta^{18}O$ method can be used as an independent method to determine the age and the timing of the formation of external rings for *Placopecten magellanicus*.

The difference in our results and those of Krantz et al. (1984) suggests that for different areas, an independent method, such as isotope analysis, should be used to verify that the rings are annual events. This implies that having a reader that is familiar with shells from a specific area may greatly improve accuracy when ageing shells by counting "annual" rings.

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Long-Term Sublethal Acid Exposure in Rainbow Trout (*Salmo gairdneri*) in Soft Water: Effects on Ion Exchanges and Blood Chemistry

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Audet, C., R. S. Munger, and C. M. Wood. 1988. Long-term sublethal acid exposure in rainbow trout (*Salmo gairdneri*) in soft water: effects on ion exchanges and blood chemistry. Can. J. Fish. Aquat. Sci. 45: 1387–1398.

Long-term sublethal acid exposure (3 mo, pH 4.8) in adult rainbow trout (*Salmo gairdneri*) acclimated to artificial soft water (Ca^{2+} = 50, Na^+ = 50, Cl^- = 100 μeq·L^{-1}) caused transient net losses of Na^+ and Cl^-. Net flux rates of both ions were returned to control levels after 30–52 d of acid exposure through a new equilibrium between unidirectional influx and efflux, where both were lower than control rates. K^+ balance remained negative and Ca^{2+} balance at zero throughout the exposure. No changes in net acidic equivalent flux occurred, indicating the absence of acid–base disturbance, but ammonia excretion increased over time. Muscle K^+, Na^+, and Cl^- fell and Ca^{2+} increased. Plasma Na^+, Cl^-, and osmolality decreased, while plasma protein, glucose, and blood hemoglobin increased during the first few weeks of acid exposure. Plasma K^+ and Ca^{2+} did not change. General stabilization of plasma parameters occurred in concert with the stabilization of Na^+ and Cl^- flux rates, but no recovery to control levels was observed for any of them. We conclude that despite this stabilization at a new steady state, rainbow trout were physiologically affected in a deleterious manner by chronic sublethal acid exposure in soft water.

Chez la truite arc-en-ciel (*Salmo gairdneri*) acclimatée à de l'eau douce à faible teneur ionique (Ca^{2+} = 50, Na^+ = 50, Cl^- = 100 μeq·L^{-1}), l'exposition prolongée à pH acide sous-létal (3 mo, pH 4,8) a causé, dans un premier temps, des pertes nettes de Na^+ et de Cl^-. Les flux nets des deux ions ont été rétablis après 30 à 52 jours d'exposition, ceci à travers un nouvel équilibre des flux unidirectionnels d'entrée et de sortie, les deux étant plus faibles que chez les contrôles. Le flux net de K^+ s'est maintenu négatif et celui de Ca^{+2} près de zéro tout au long des 3 mo d'exposition. Les flux nets d'équivalents acides n'ont pas été affectés, indiquant que l'exposition à l'acidité n'a pas créé de déséquilibre acido-basique. Cependant, l'excrétion d'ammoniaque a augmenté en fonction du temps d'exposition. Dans le muscle, on a observé des pertes de K^+, de Na^+ et de Cl^- mais une augmentation du Ca^{+2}. Durant les premières semaines d'exposition, le Na^+, le Cl^- et l'osmolalité plasmatiques ont décru alors que les concentrations plasmatiques de protéines, de glucose et la concentration d'hémoglobine sanguine augmentaient. Le K^+ et le Ca^{+2} plasmatiques n'ont pas été affectés. Plusieurs paramètres plasmatiques se sont stabilisés de concert avec la stabilisation des flux de Na^+ et de Cl^- mais aucun retour aux niveaux contrôles n'a été observé. Nous concluons, qu'en dépit du fait qu'un nouvel équilibre physiologique ait été atteint durant l'exposition prolongée à un pH acide sous-létal, la truite arc-en-ciel est physiologiquement affectée par l'acidité lorsque ce stress est appliqué en eau douce à faible teneur ionique.

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Acidification of freshwater habitats in Canada, as well as in numerous other northern countries, now endangers many natural fish populations. There have been many studies on the toxic *lethal* effects of acute acid exposure in fish. In salmonids, it is now generally recognized that the toxic mechanism of acute lethal exposure (pH < 4.5) results from ionoregulatory disturbance (reviewed by Leivestad et al. 1976; Muniz and Leivestad 1980; Wood and McDonald 1982; Howells et al. 1983; McDonald 1983a; Wood 1988a). This conclusion may only be true when fish are held in soft water (low ionic strength and calcium content), but such conditions are representative of freshwater habitats threatened by the acidification process.

On the other hand, the effects of chronic *sublethal* acid exposure (pH 4.5–6.0) are not so well known, and no clear pattern has yet emerged. Some studies seem to indicate that adaptation to such an environment is possible (e.g. McWilliams 1980; Fraser and Harvey 1984; Leino and McCormick 1984; Wood et al. 1988a; Sadler and Lynam 1986), while others have demonstrated chronic ionoregulatory disturbance, growth impairment, increased levels of stress indicators, and/or a reduction of reproductive capacity (e.g. Menendez 1976; Lee et al. 1983; Saunders et al. 1983; Brown et al. 1984; Giles et al. 1984; Johnston et al. 1984; Rodgers 1984; Haya et al. 1985; Lacroix 1985; Scherer et al. 1986; Tam and Payson 1986; Weiner et al.

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1986; Jones et al. 1987; Lacroix and Townsend 1987; Tam et al. 1987; Wood et al. 1988c). The situation is further complicated by the fact that only a few of these studies (Saunders et al. 1983; Fraser and Harvey 1984; Haya et al. 1985; Johnston et al. 1984; Lacroix 1985; Weiner et al. 1986; Lacroix and Townsend 1987; Wood et al. 1988a, 1988c) were performed in soft water (i.e. $[Ca^{2+}] < 200$ $\mu eq \cdot L^{-1}$); the remainder were performed in either hard water or water of unstated composition.

In view of the fact that large areas of natural soft water in eastern Canada are now acidified to the pH 4.5–6.0 range (Kelso et al. 1986; Minns and Kelso 1986), there is a clear need to improve our knowledge on the physiological effects of long-term sublethal acid exposure in soft water. The work presented here is part of an overall study on the ionoregulatory, endocrine, and branchial morphological responses of adult rainbow trout (*Salmo gairdneri*) to long-term (12 wk) sublethal acid exposure (pH 4.8) in flowing soft water. The artificial soft water used was chosen to duplicate the composition of typical acid-threatened lakes on the Canadian Shield. The present paper describes unidirectional Na^+ and Cl^- fluxes and net fluxes of Na^+, Cl^-, Ca^{2+}, and K^+, titratable acid, ammonia, and acidic equivalents measured periodically in the same animals throughout the 12 wk of exposure. The goal was to test whether ionoregulatory disturbance, and not acid–base disturbance, was the major consequence of long-term sublethal acid exposure, as is the situation during short-term lethal exposure (see Wood 1988a for review), and to characterize the nature of any disturbances which occurred. We also looked at the general internal physiological status of these fish to see if physiological steady state was achieved during chronic acid exposure.

Methods

Fish Holding and Acid Exposure

Adult rainbow trout (200–300 g) of both sexes were obtained from a hardwater source (Spring Valley Trout Farm, Petersburg, Ontario) and initially held in Hamilton tap water (Ca^{2+} = 1800, Na^+ = 600, Cl^- = 800 $\mu eq \cdot L^{-1}$). Stock and experimental fish were kept at 15 ± 1°C under a 24-h light photoperiod. The fish were first acclimated for 2–4.5 mo to artificial soft water (ASW: Ca^{2+} = 50, Na^+ = 50, Cl^- = 100 $\mu eq \cdot L^{-1}$; pH = 6.5) before being used as either controls (no acid exposure) or experimentals (chronic acid exposure). None of the fish were in breeding condition.

ASW was generated by dechlorination of tap water, followed by deionization using a reverse osmosis system (Culligan Aqua-Clear MP1000), and final supplementation of the product with $CaCl_2$ and NaCl stock solutions via a peristaltic pump. Approximately 30 fish were kept in a 450-L tank during ASW acclimation and during each experiment. ASW was delivered via a continuous flowing system providing 90% replacement of the tank volume every 24 h, as calculated from the nomogram of Sprague (1973). The fish were fed once a week to satiation with trout pellets (40% floating trout feed grower pellets, Martin Feed Mills Limited). In view of the limited volume of our ASW supply, this feeding regime was selected as one adequate to maintain body weight (see Results) while minimizing contamination of the holding water. The time of feeding was not kept constant relative to sampling periods, but the fish were not fed in the 48 h prior to any experiment or sampling.

After at least 2 mo of acclimation to ASW at pH ≈ 6.5, the pH was lowered to 4.8. Acidified water was obtained by titration of the flowing soft water with 0.5 N H_2SO_4 using a Radiometer titrator (TT80), pH meter (pHM 82), and combination glass-reference electrode (GK2401C) which opened and closed a magnetic valve to deliver acid to a header tank. The soft water in the header tank was vigorously bubbled with air and passed through a gas stripping column prior to entering the fish holding tank in order to prevent P_{CO_2} elevations. Water pH in the holding tank was continuously recorded on a chart recorder via an independent pH meter (Fisher 119) coupled to a Cole-Palmer (5658-10) combination glass-reference electrode. Water samples were taken weekly during the two first experiments and daily for the other three to monitor Na^+ and Ca^{2+} levels. Control ASW pH varied within ±0.3 unit, acidified ASW pH within ±0.1 unit, and Ca^{2+} and Na^+ concentrations within ±9 $\mu eq \cdot L^{-1}$.

Experimental Design

The basic experimental protocol of 2–4.5 mo of ASW acclimation at pH ≈ 6.5 followed by up to 12 wk of pH 4.8 exposure was repeated five times in experiments starting November 1985, April 1986, October 1986, January 1987, and June 1987. This repetition of experiments was necessary because of our limited supply of ASW. The first experiment focussed on repetitive flux measurements in the same 12 fish throughout the 12-wk period (control, 5 h, and 1, 2, 4, 7, 11, 16, 23, 30, 52, and 81 d). Terminal blood samples were taken on day 81. Other fish from this same experiment were utilized in the challenge experiment described by Audet and Wood (1988) after day 81. The other series provided fish for terminal blood and tissue sampling before (control) and at various times during the 12-wk exposure (4 h and 1, 3, 8, 22, 50, and 81 d). Not all experimental times were sampled in each series, but control samples were taken in every experiment. As expected, given the constant temperature and photoperiod regimes, no significant differences among series were found in controls or at common sample times. Therefore the data from the five series were pooled.

A simultaneous exposure of the fish to ASW at neutral pH was not run in parallel to each of the series, in view of the relatively long period allowed for ASW acclimation, and the fact that the experiment was repeated five times. We kept control fish for period varying from 2 to 4.5 mo in ASW and no significant differences were found between our five groups. While trout do not acclimate instantaneously to soft water, adjustment is complete within several weeks (McDonald et al. 1980; McDonald and Rogano 1986). Therefore, we did not expect that additional weeks in ASW would bring any significant changes in the status of the fish.

Flux Measurements

In order to minimize variation and perform accurate backflux correction of the radioisotopic uptake (see below), it was essential that the same 12 fish be tested at each time of flux measurements. Thus the trout used for these measurements were individually coded for identification with plastic wires anchored through the dorsal fin.

At least 24 h prior to a flux determination, the fish were transferred to individual flux boxes of the type described by McDonald (1983b). These consisted of a transparent inner chamber which confined the fish and a dark outer one (with transparent lid) which contained the majority of the water volume (≈7 L). An airlift pump at the rear of the inner chamber recirculated the water and maintained oxygen saturation. Mix-

ing was further aided by perimeter aeration of the outer box. During the adjustment period, each box received a constant flow of water (1.5 L·min^{-1}) at the appropriate pH. All flux measurements were performed at times between 1100 and 1600 to minimize the influence of diurnal rhythms. At the beginning of the experiments, flow of water through the box was stopped (aeration and temperature being maintained), the volume set to a precise figure, and 5 μCi (185 kBq) of $^{22}Na^+$ and 5 μCi of $^{36}Cl^-$ (New England Nuclear) added to each box. We allowed 30 min for the mixing of radioisotopes and then started sampling (time 0). Every hour for 5 h, 60 mL of water was sampled from each box. From these 60 mL, different aliquots were prepared: 10 mL for titratable alkalinity (kept at 5°C and analyzed within 12 h), 2 × 5 mL for gamma counting, 2 × 5 mL for scintillation counting, and 10 mL to which we added one drop of concentrated Aristar HNO_3 before storage at 5°C for subsequent Cl^- and cation measurements. The rest of the water was used for immediate pH measurement (Radiometer PHM82 meter plus GK2401C electrode), frozen at −20°C, and later used for ammonia determination. The water pH of each box was therefore maintained at the experimental value by adding an appropriate volume of 0.02 N H_2SO_4 once every hour. All flux measurements were based on average values for the 5 h of each measurement period.

The net fluxes (J_{net}) of Na^+, Cl^-, K^+, and Ca^{2+} were given by the changes of the total amount of ions in the water volume. J_{in} for Na^+ and Cl^- was measured by the disappearance of radioisotopes from the water, while J_{out} for Na^+ and Cl^- was given by the subtraction of J_{in} from J_{net}. Net losses by the fish have a negative sign and net gain a positive sign. The different equations were as formulated in Hōbe et al. (1984) except for J_{in}, as we had to apply a significant backflux correction. As the same fish were subjected to radioisotope flux measurements 12 times during the 81-d exposure, they accumulated significant amounts of radioactivity internally. The efflux of this radioactivity ("backflux") during subsequent flux measurement periods would thereby tend to reduce the calculated J_{in} values below real values. We therefore applied the backflux correction equation of Maetz (1956):

$$(1)\quad J_{in} = \frac{V_{ext}\cdot((C_i - C_f) - SA_{int}\cdot(X_i - X_f))}{SA_{ext}\cdot W\cdot t}$$

where C_i and C_f are initial and final radioactivities in the water (counts per minute (cpm) per millilitre) during the flux period in question, X_i and X_f are the initial and final concentration in the water (microequivalents per millilitre) of the ion in question, SA_{int} and SA_{ext} are the mean internal and external specific activities (cpm per microequivalent) over the period (see equations 2 and 3), V_{ext} is the volume of the system (millilitres) corrected for sampling deficits, t is the elapsed time (hours), and W is the body weight (kilograms). SA_{ext} and SA_{int} are given by

$$(2)\quad SA_{ext} = \frac{1}{2}\cdot\left(\frac{C_i}{X_i} + \frac{C_f}{X_f}\right)$$

$$(3)\quad SA_{int} = \frac{\Sigma C_i + \Sigma C_f}{2\cdot V_{int}\cdot X_p}$$

where ΣC_i and ΣC_f are the summated $^{22}Na^+$ or $^{36}Cl^-$ radioactivity (cpm) accumulated by the fish from the water at the start and end of the flux period, respectively, V_{int} is the internal radiospace, and X_p is the plasma concentration of the ion in question. V_{int} was taken as 280.35 mL·kg^{-1} for Na^+ and 252.65 mL·kg^{-1} for Cl^-, from the measurements of Wood (1988b) in rainbow trout. Plasma ion levels for each fish at any time were based on regression lines between the control value and the measured terminal value.

In order to estimate ΣC_i and ΣC_f, it was necessary to calculate the amount of radioactivity lost since the previous determination. We used the formula

$$(4)\quad \text{cpm lost} = (\text{cpm}\cdot\text{fish}^{-1})\cdot e^{(-J_{out}^{Na^+}\cdot W\cdot SA_{int}\cdot h)}$$

where cpm·fish^{-1}, $J_{out}^{Na^+}$, and SA_{int} were as measured at the end of the previous flux period.

At the end of the experiment (day 81), we measured SA_{int} for Na^+ and Cl^- in the terminal plasma samples for comparison with the value calculated by the above procedures (equations 3 and 4). For $SA_{int}^{Na^+}$, we did not find any difference between the calculated and the measured values. However, we underestimated the $SA_{int}^{Cl^-}$, calculated values being 56% of measured. Model calculation based on these discrepancies indicate that the maximum possible error would have occurred on day 16 (8% underestimate of Cl^- influx); errors on other days would have been far less. We therefore consider these possible errors relatively unimportant.

Water cations (Na^+, K^+, and Ca^{2+}) were measured by atomic absorption (Varian 1275-AA). Cl^- was measured by coulometric titration (Buchler chloridometer) or by a colorimetric method based on the formation of ferric thiocyanate proportional to the chloride concentration in the water sample (Zall et al. 1956). $^{22}Na^+$ was measured by gamma counting (Nuclear Chicago model 1085), and combined $^{22}Na^+$ and $^{36}Cl^-$ cpm were determined by liquid scintillation counting (LKB 1217, Rackbeta). $^{36}Cl^-$ cpm were obtained by subtraction after correcting for differences in efficiency of $^{22}Na^+$ counting by the two instruments.

Total ammonia measurements utilized the salicylate–hypochlorite reaction of Verdoux et al. (1978). Titratable alkalinity was measured as previously described by McDonald and Wood (1981). Values were corrected for 0.02 N H_2SO_4 addition to the flux boxes during the experiments. The net titratable acid flux was calculated from the change in titratable alkalinity. The net flux of acidic equivalents was calculated as the sum of the net titratable acid and the net ammonia flux, signs considered. As McDonald and Wood (1981) pointed out, the method does not distinguish between ammonia measurement in the NH_3 and NH_4^+ forms, nor between the net excretion of acidic equivalents and the net uptake of basic equivalents, or vice versa. Fortunately, this does not matter in terms of the net acid–base budget of the fish.

Blood and Tissue Chemistry

Blood and tissue samples at various times during the 12-wk exposure were taken from fish in the other four experimental series and on day 81 from the first series. Each experiment started with about 30 fish. At sampling, fish were individually anesthetized in 0.01% MS-222 (pH adjusted to 6.5 or 4.8 as appropriate with KOH), and blood was withdrawn immediately by caudal puncture. At some sample times (control and 1, 3, 8, and 81 d), white muscle samples were also excised from the epaxial mass below the dorsal fin. All samples were taken at times between 1330 and 1700 to minimize the influence of diurnal rhythms.

Plasma was separated by centrifugation (10 000 × g for 2 min). Plasma cations (Na^+, K^+, and Ca^{2+}) were measured

by atomic absorption (Varian 1275-AA), Cl^- by coulometric titration (Radiometer CMT10 chloridometer), osmolality by vapor pressure osmometry (Wescor 5100B), and plasma proteins by refractometry (American Optical TS meter). The calibration of the refractometer had earlier been validated against the Biuret method for plasma protein (Sigma procedure No. 540). Hemoglobin and glucose were measured using commercial kits (Sigma procedures No. 525 and No. 16-UV). Hematocrit was measured by centrifugation (5000 × *g* for 5 min) and mean cell hemoglobin concentration (MCHC) as the ratio of hemoglobin concentration to hematocrit.

Muscle water content was determined by drying to a constant weight at 85°C. The same tissue samples were subsequently analysed for Na^+, K^+, Ca^{2+}, Cl^-, and Mg^{2+} by instrumental neutron activation using the facilities of the McMaster Nuclear Reactor (cf. Spry et al. 1988 for methodology).

Statistical Analysis

In the flux series, for which the same 12 fish were always used, the number of fish decreased from 12 to 7 due to mortality in the two last sampling periods (52 and 81 d). However, for the sampling days preceding day 52, we did not find any significant differences between the fish which survived through the end of the experiment versus the ones that died (*t*-test). The data from all fish were therefore pooled and used for subsequent analysis.

Data have been routinely expressed as mean ± SEM (*N*), where *N* represents the number of fish. All the data were analysed by nonbalanced one-way ANOVA, $p \leq 0.05$ (time of exposure tested as the source of variation), followed by a Tukey–Kramer test of comparison of means (Sokal and Rohlf 1981) with $\alpha = 0.05$. Normality of the data and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests, respectively. All the data were normally distributed; however, in some cases, transformations were required to obtain homogeneity of variances. Thus, data for $J_{out}^{Cl^-}$ and titratable acid flux were transformed as $(x + 325)^2$ and $\log_{10}(x)$, respectively, plasma Cl^- and plasma glucose as x^4 and $1/x$, respectively, and data for muscle Ca^{2+} and K^+ as $\log_{10}(x)$ and x^4, respectively, prior to ANOVA. Arithmetical means ± SEM are shown on the figures. All tried transformations failed to obtain homogeneity of variances for $J_{net}^{Ca^{2+}}$, acidic equivalents flux, and muscle Na^+. In these cases, ANOVA was applied for overall effects and then the Games and Howell test of comparison of means, designed for heterogeneous variances, was applied according to Sokal and Rohlf (1981).

Results

Mortalities

From the 12 rainbow trout used for flux measurements in the first experimental series, 4 died between the fourth and the seventh week of acid exposure and 1 more between the seventh and the twelfth week. Five other fish from the first series died between the fourth and the twelfth week. There were no mortalities in the subsequent four experimental series.

Ion Exchanges

The main effect of sublethal acid exposure (pH 4.8) in adult rainbow trout was a transient ionoregulatory disturbance reflected in net losses of both Na^+ and Cl^- (Fig. 1). ANOVA showed that J_{in} and J_{out}, as well as J_{net}, for both Na^+ and Cl^- were influenced by the length of sublethal acid exposure. Net Na^+ and Cl^- fluxes became negative and significantly different from the control levels on the first day of exposure, but were completely restored after 30–52 d of acid exposure. However, this was achieved through a new equilibrium between J_{in} and J_{out}, rather than by a return of J_{in} and J_{out} to control levels.

Disturbance of $J_{net}^{Na^+}$ occurred very rapidly (Fig. 1a), with a changeover to negative balance in the first 5 h of acid exposure. The net loss of Na^+ was the direct consequence of a 70% inhibition of J_{in} over first 16 d of exposure. A partial recovery of $J_{in}^{Na^+}$ subsequently occurred, but $J_{in}^{Na^+}$ was still significantly below the control values even after 81 d of exposure. Net Na^+ loss caused by this inhibition of $J_{in}^{Na^+}$ was partly compensated by a ≈40% decrease of $J_{out}^{Na^+}$ (Fig. 1a). J^{Na^+} was significantly reduced in the first 5 h of acid exposure without any subsequent significant recovery over 81 d of acid exposure. The recovery of Na^+ net flux observed during chronic acid exposure was therefore the result of a partial recovery of $J_{in}^{Na^+}$ coupled with a persistent inhibition of $J_{out}^{Na^+}$.

Overall variations of $J_{net}^{Cl^-}$ were similar to those observed for Na^+ (Fig. 1b). Significant reduction occurred by 24 h and $J_{net}^{Cl^-}$ flux remained negative through 23 d of acid exposure. At day 30, net flux became positive and no longer different from the control. The negative net flux was the result of a significant inhibition of $J_{in}^{Cl^-}$ (Fig. 1b). Within 5 h of exposure, $J_{in}^{Cl^-}$ was reduced by half and was kept around 30% of the control value during the first days of exposure. Recovery occurred gradually and $J_{in}^{Cl^-}$ was equal to about 75% of control value from 30 d of exposure through the end of the experiment. Changes in $J_{out}^{Cl^-}$ (Fig. 1b) were less marked than in $J_{out}^{Na^+}$, a significant reduction from control value being observed only at 2, 16, and 81 d of chronic acid exposure.

No significant variations were observed for either Ca^{2+} or K^+ net fluxes. On average, fish were in balance for their Ca^{2+} exchange with the environment, overall mean Ca^{2+} fluxes being equal to 1.2 ± 3.94 $\mu eq \cdot kg^{-1} \cdot h^{-1}$ (133). The overall mean of K^+ fluxes was equal to -15.1 ± 0.9 $\mu eq \cdot kg^{-1} \cdot h^{-1}$ (134). There was no major effect of acid exposure on net fluxes of acidic equivalents (Fig. 2). Overall ANOVA indicated a significant treatment effect but the a posteriori test showed a significant difference only between day 81 and day 2, with neither being different from the control value. Although the net balance of acidic equivalents was little affected, this was not true for the separate components of that measurement. Thus, ammonia excretion continuously rose with time, increasing by 75% after 81 d of acid exposure compared with the control value (Fig. 2). The story for titratable acidity was less clear although the general trend was similar to that for ammonia excretion. A transient decrease of titratable acid uptake was found at 2 d. Significant increases occurred after 52 and 81 d of acid exposure.

Blood and Tissue Chemistry

The plasma ion data confirmed the results of whole-body flux measurements. Almost all measured parameters were affected by chronic acid exposure, but except for plasma glucose levels, all these deviations from control values stabilized before the end of the experiment. However, in no case was there any recovery to the control values.

Plasma Na^+ and Cl^- levels underwent major changes during chronic acid exposure. After 8 d, plasma Na^+ concentration was significantly reduced, and stabilized around 86% of the control value after 22 d of acid exposure (Fig. 3a). A similar

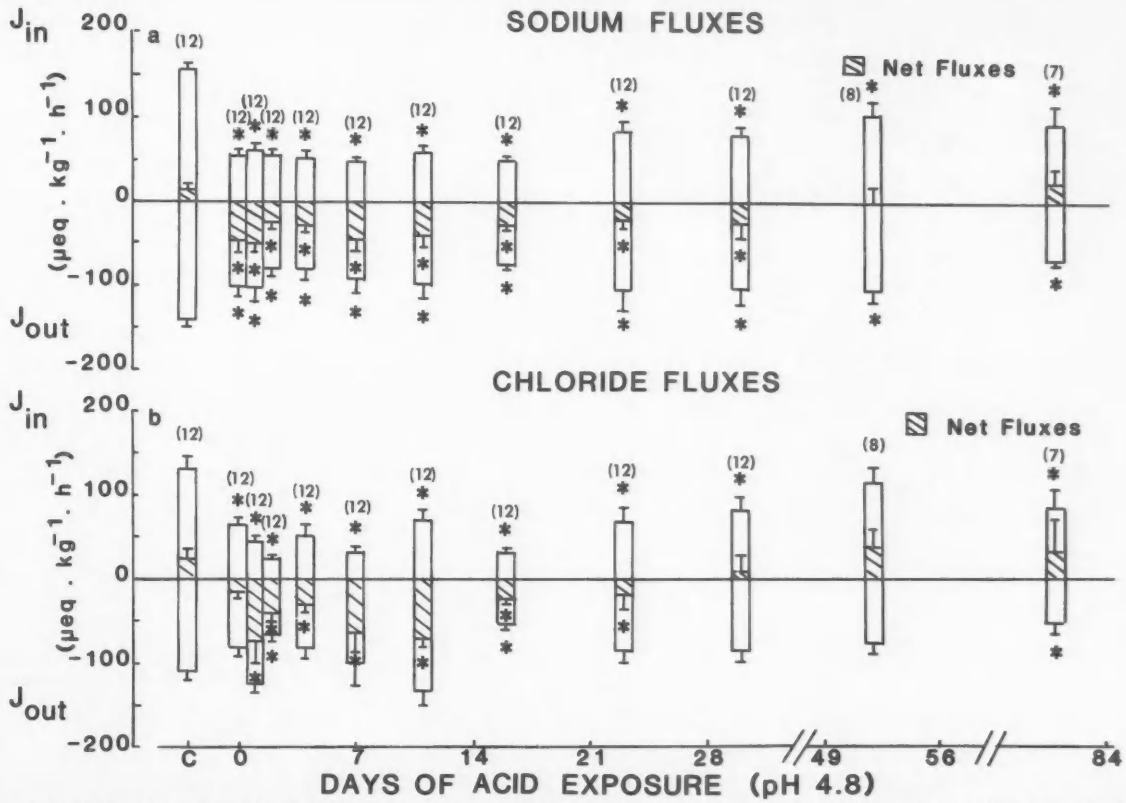

FIG. 1. (a) Sodium and (b) chloride fluxes during long-term sublethal acid exposure in rainbow trout. Significant effects of the length of acid exposure occurred for each set of data (ANOVA, $p \leq 0.01$: $J_{net}^{Na^+}$, $J_{in}^{Na^+}$, $J_{net}^{Cl^-}$, $J_{in}^{Cl^-}$, and $J_{out}^{Cl^-}$; $p \leq 0.05$, $J_{out}^{Na^+}$). Means ± SEM; numbers in parentheses indicate the number of fish; asterisks indicate the days of exposure for which the results are significantly different from the control values. The complete results of the mean comparison test are presented below. The numbers represent the days of exposure, and single lines underscore days between which there was no significant difference ($p \leq 0.05$). C = control experiment prior to acid exposure.

$J_{net}^{Na^+}$ 1 0 7 11 4 16 2 30 23 52 C 81

$J_{in}^{Na^+}$ 7 16 4 0 2 11 1 30 23 81 52 C

$J_{out}^{Na^+}$ C 23 52 30 1 0 11 7 4 3 16 81

$J_{net}^{Cl^-}$ 1 11 7 2 4 16 23 0 30 C 81 52

$J_{in}^{Cl^-}$ 2 16 7 1 4 23 11 0 30 81 52 C

$J_{out}^{Cl^-}$ 11 1 C 30 7 4 23 0 52 2 16 81

pattern occurred for plasma Cl^- (Fig. 3b). The final Cl^- decrease was slightly greater (around 20% of the initial level) than for Na^+. Plasma levels of Ca^{2+} (Fig. 3c) were not affected by chronic acid exposure, while only a transient increase of plasma K^+ (Fig. 3c) was observed during the first hours of acid exposure.

A short-term 5% decrease of plasma osmolality occurred in the first hours of acid exposure (Fig. 4a). A significant drop of similar magnitude was also observed after 8 d. Thereafter, osmolality remained constant at a level about 20 mosm·kg^{-1} below control. This was a smaller relative and absolute decrease than in plasma Na^+ and Cl^- levels (Fig. 3a, 3b). The plasma protein concentration changed in the opposite direction (Fig. 4b). From day 1 onwards, plasma protein levels were significantly elevated by 20–30% throughout the period of acid exposure, except at day 50 where the results were anomalous, perhaps due to the low sample number. Plasma glucose increased dramatically over time, being significantly different from the control group after only 4 h of exposure and reaching threefold to fivefold the control level at days 22–81 (Fig. 4c).

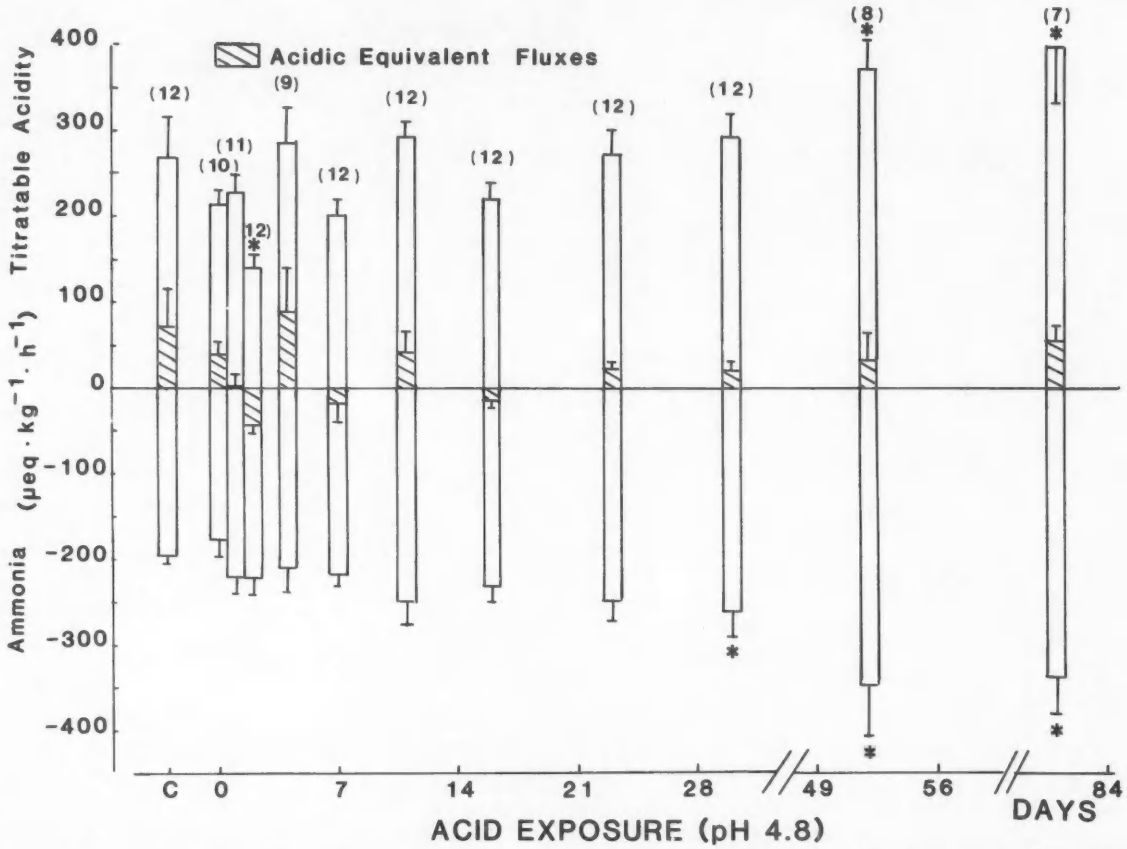

FIG. 2. Net acidic equivalent, ammonia, and titratable acidity fluxes during long-term sublethal acid exposure in rainbow trout. Significant effects of length of acid exposure were found for each parameter (ANOVA, $p \leq 0.01$). See legend to Fig. 1 for other details.

$J_{net}^{Amm.}$ 52 81 30 11 23 16 2 1 7 4 C 0

$J_{net}^{Tit.\ ac.}$ 2 7 16 0 1 C 30 23 4 11 52 81

J_{net}^{H+} 2 7 16 1 30 23 52 11 0 81 C 4

Hemoglobin concentration in the blood increased by about 25% under chronic acid exposure (Fig. 5a). A first increase was observed at day 3 and a second one at day 22 after which hemoglobin concentration remained relatively constant through the end of the experiment. Rather surprisingly, there were no significant effects on hematocrit (Fig. 5b) which remained stable at an overall average of 36.0 ± 0.8% (90) through the whole experiment. The net result of these changes was an increase of the MCHC index during chronic acid exposure (Fig. 5c). Such changes in MCHC suggest either a shrinking of red blood cells or an increase in their hemoglobin content.

Ion concentrations in epaxial white muscle changed over time under chronic acid exposure (Table 1). The main muscle tissue electrolyte, K^+, was not affected during the first week of acid exposure but was reduced by 20% after 81 d. Mg^{2+} concentration was not affected. Significant effects of the length of exposure on muscle Na^+ and Cl^- were found, both falling by 40–50% by day 81. However, the a posteriori test demonstrated a significant difference only for Cl^-. Muscle Ca^{2+} was stable during the first week, but increased by 67% after 81 d of exposure.

No significant effects of chronic acid exposure on either weight (overall mean of 0.239 ± 0.005 kg (88)) or white muscle water content (overall mean of 79.9 ± 0.2% (50)) were observed.

Discussion

The present study is the first to assess the chronic physiological responses of adult salmonids to long-term *sublethal* acid exposure in soft water under controlled laboratory conditions. The whole-body flux measurements showed that ionoregulatory disturbance is the main toxic effect of such exposure. No disturbance of net acidic equivalent exchange with the environment is associated with such conditions, and therefore, internal acidosis should not occur. This confirms results already

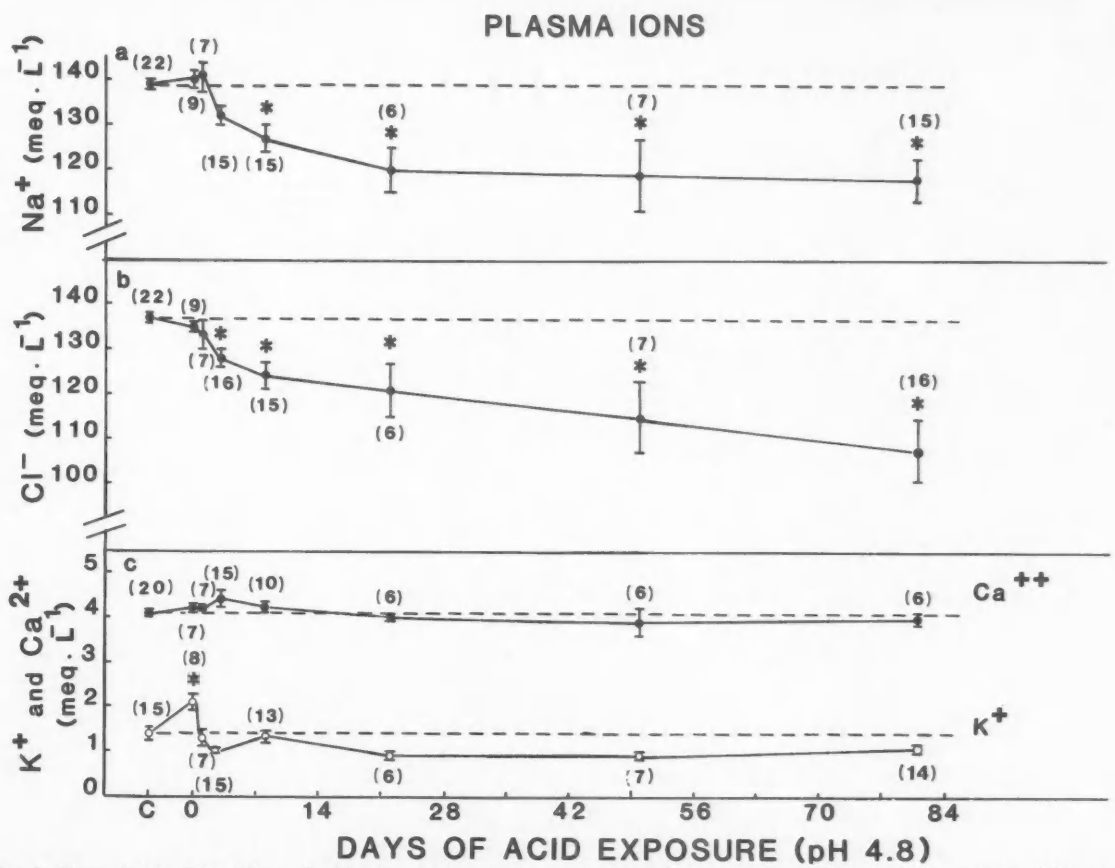

FIG. 3. Plasma levels of (a) sodium, (b) chloride, and (c) potassium and calcium during long-term sublethal acid exposure in rainbow trout. Significant effects of length of acid exposure were found for Na^+, Cl^-, and K^+ (ANOVA, $p \leq 0.01$) but not for Ca^{2+}. See legend to Fig. 1 for other details.

Plasma Na^+	50	81	22	8	3	C	0	1
Plasma Cl^-	81	50	22	8	3	1	0	C
Plasma K^+	22	50	3	81	1	8	C	0

obtained under acute *lethal* acid exposure in rainbow trout in soft water (reviewed by Wood 1988a). It has been demonstrated previously that gill fluxes account for the major part of ion losses (around 80%) during lethal exposure (McDonald and Wood 1981; McDonald 1983b; McDonald et al. 1983). Therefore, it is reasonable to believe that the major portion of this disturbance of ionic exchange during sublethal exposure is also located at the gill site.

Both the intensity and the nature of ionoregulatory disturbance observed under chronic acid exposure are different from those under acute lethal exposure in rainbow trout. Under sublethal acid exposure (pH 4.8), $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$ are only partially inhibited, while under lethal acid conditions (pH 4.0–4.5), J_{in} of both are almost completely inhibited (McDonald et al. 1983; Audet and Wood 1988). Moreover, a partial recovery of influx occurred under chronic acid exposure, and alterations in J_{out} (see below) were rather different from those seen under acute exposure to more severe pH.

While functional gill models suggest that the chloride cells are the most likely sites of active ionic uptake in freshwater fish (e.g. Evans 1982), this has remained controversial, with other investigators believing that the lamellar respiratory cells are the important transporting cells (e.g. Girard and Payan 1980; Payan et al. 1984). However, more recent work points strongly to the chloride cells as the major sites of active uptake (Perry and Wood 1985; Avella et al. 1987). Na^+/NH_4^+,H^+ and Cl^-/HCO_3^-,OH^- exchanges are thought to occur at the apical border of these cells. It has been proposed (reviews by McDonald 1983a; Wood 1988a) that inhibition of sodium uptake during acid exposure could be the result of a H^+ competition with Na^+ for the transport site and/or a titration of negative charges in Na^+-specific channels, thereby restricting access of the ion to its transport system. By either mechanism, the inhibition of Na^+ uptake would be concentration dependent, thereby explaining the only partial effect of pH = 4.8 relative to more complete inhibition in previous studies at pH = 4.0–

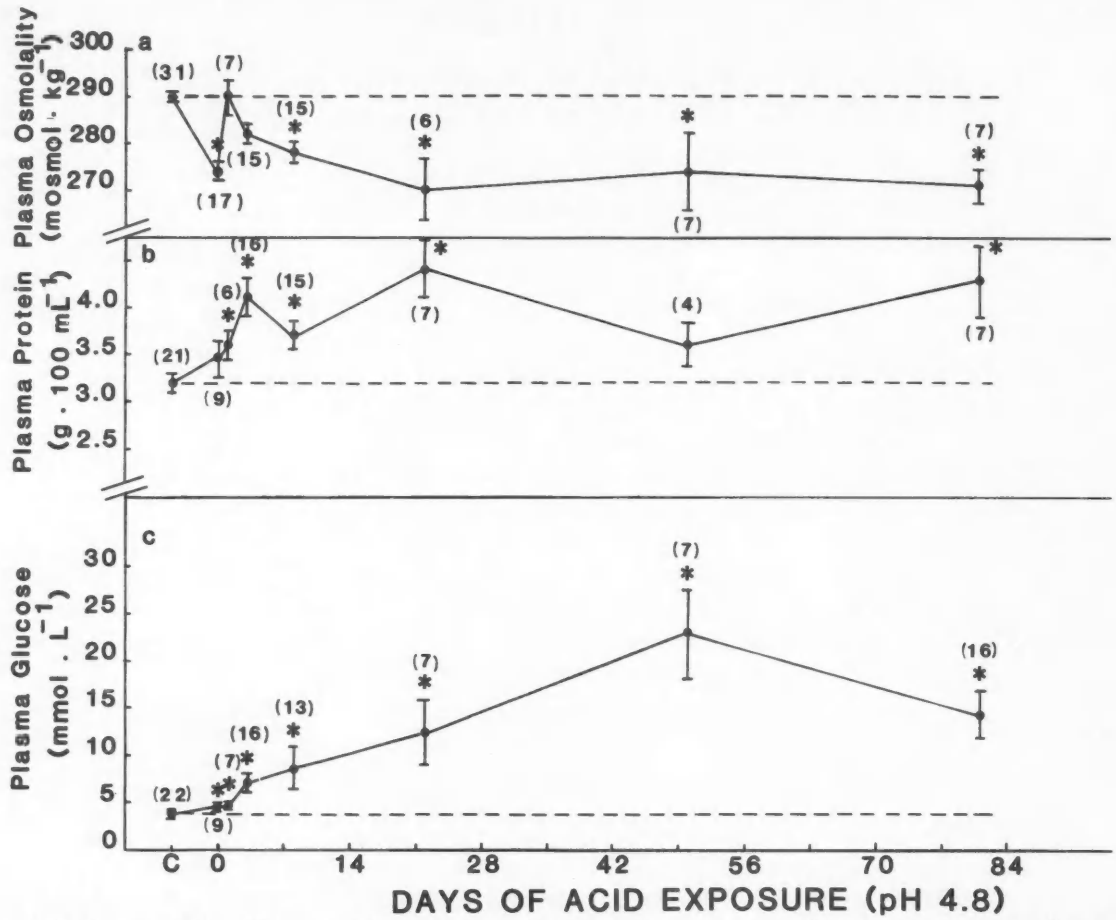
Plasma Osmolality (mosmol · kg⁻¹)
Plasma Protein (g · 100 mL⁻¹)
Plasma Glucose (mmol · L⁻¹)
a
b
c
DAYS OF ACID EXPOSURE (pH 4.8)
C 0 14 28 42 56 70 84

FIG. 4. (a) Plasma osmolality, (b) plasma protein concentration, and (c) plasma glucose concentration during long-term sublethal acid exposure in rainbow trout. Significant effects of length of acid exposure were found for all parameters (ANOVA, $p \leq 0.01$). See legend to Fig. 1 for other details.

Plasma osmolality	22	81	0	50	8	3	C	1
Plasma protein	C	0	50	1	8	3	81	22
Plasma glucose	50	81	22	8	3	1	0	C

4.5. Unfortunately, no satisfactory explanation is yet available for the inhibition of chloride uptake at low pH.

The partial recovery of Na^+ and Cl^- uptake during chronic exposure is particularly interesting. McWilliams (1980) reported a complete recovery of $J_{in}^{Na^+}$ in brown trout (*Salmo trutta*) chronically exposed to a very mild acidity (pH = 6.0) in hard water. Possible explanations include adaptative changes in the affinities of the carriers for Na^+ and Cl^- and/or increases in the amount of carrier available. The recent study of Avella et al. (1987) on rainbow trout suggests that $J_{in}^{Na^+}$ is closely correlated with the abundance of chloride cells in the gills. Leino and McCormick (1984) reported both a proliferation of chloride cells and a change in chloride cell morphology during chronic exposure of fathead minnows (*Pimephales promelas*) to pH = 5.0–5.5 in moderately hard water. It is possible that a similar phenomenon was responsible for the partial recovery of $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$ in the present study. Nevertheless, the recovery was insufficient to restore the internal levels of these ions, which at best were stabilized, not corrected.

An important observation is the ability of the fish to reduce $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ immediately and maintain this reduction during chronic sublethal acid exposure. This is different from acute acid stress where net Na^+ and Cl^- losses were mainly the consequence of tremendous initial increases of J_{out} which then decreased over time (for a review, see Wood and McDonald 1982; Wood 1988a). The decrease of J_{out} observed under sublethal acid exposure could be the consequence of a decrease of membrane permeability through endocrine modulation. Wen-

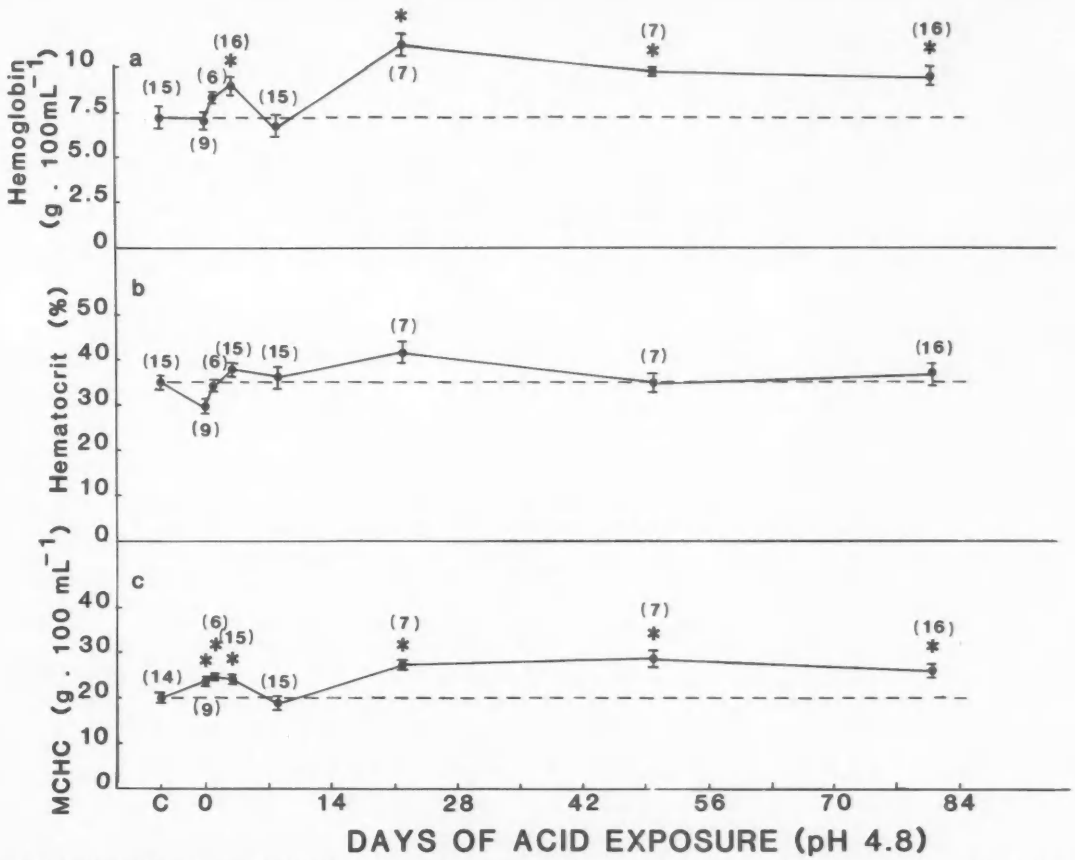

FIG. 5. Blood hematology during long-term sublethal acid exposure in rainbow trout, (a) Hemoglobin concentration; (b) hematocrit; (c) MCHC. Significant effects of length of acid exposure were found for hemoglobin and MCHC (ANOVA, $p \leq 0.01$) but not for hematocrit. See legend to Fig. 1 for other details.

Hemoglobin	8	0	C	1	3	81	50	22
MCHC	8	C	0	3	1	81	22	50

TABLE 1. Ion concentrations (meq·kg^{-1} wet weight) in muscle tissue of rainbow trout during long-term sublethal acid exposure. Means ± SEM; number of fish was always 7; mean values from the same column but not followed by a common letter were significantly different (ANOVA, $p \leq 0.05$; Tukey test, $\alpha = 0.05$).

Length of acid exposure (d)	K^+	Mg^{2+}	Na^+	Cl^-	Ca^{2+}
Control	136.0 ± 1.96 ab	24.0 ± 0.54 a	7.8 ± 0.42 a	10.5 ± 0.60 a	4.3 ± 0.61 a
1	140.5 ± 2.54 a	23.7 ± 0.28 a	6.5 ± 0.43 a	8.9 ± 0.43 ab	4.5 ± 0.35 a
3	127.3 ± 1.49 bc	24.6 ± 0.49 a	6.2 ± 0.44 a	7.7 ± 0.42 bc	4.8 ± 0.59 a
8	132.1 ± 3.17 ab	23.9 ± 0.59 a	6.1 ± 0.15 a	8.3 ± 0.44 ab	4.2 ± 0.30 a
81	109.0 ± 8.57 c	22.4 ± 1.45 a	4.9 ± 1.01 a	5.5 ± 1.04 c	11.4 ± 1.75 b

delaar Bonga et al. (1984a, 1984b) reported that tilapia exhibited an increased synthetic activity of prolactin cells following acute acid exposure. The principal effect of prolactin in freshwater fish is a decrease of cell membrane permeability to water and ions in integuments (see Nicoll 1981 for review). McWilliams (1980) also suggested, based on changes in transepithelial potential measurements, that the success of brown trout to acclimate to a mildy acidic environment should be largely dependent on reduced gill membrane permeability.

There is an alternative hypothesis to explain the decrease of $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ in response to sublethal acid exposure. de Renzis (1975) and Wood et al. (1984) proposed that some Cl^-/Cl^- and Na^+/Na^+ exchanges (exchange diffusion) are present in the gill transport cells. Inhibition of $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$

associated with an equal decrease in $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ could reflect an inhibition of the exchange diffusion component (Na^+/Na^+ and Cl^-/Cl^-) at the gills by external acidity. By this scenario, the fraction of $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$ which persisted, and which later showed partial recovery, would be largely as Na^+/NH_4^+, H^+ and Cl^-/HCO_3^-, OH^- exchanges, respectively, while the fraction of $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ which persisted would occur largely by simple diffusion. More detailed kinetic, endocrine, and structural work will be required to determine whether either or both of these hypotheses (permeability reduction and/or exchange diffusion inhibition) are correct.

No major changes in net acidic equivalent flux were found during acid exposure. This confirms that acidosis is not a problem for fish under sublethal acid stress in ASW typical of natural acidic waters (e.g. Wood 1988a). By strong ion difference theory (Stewart 1983), acidic equivalent uptake is a dependent variable constrained by an excess of strong cation loss over strong anion loss. In rainbow trout, this occurs only during acid stress in water of high calcium content (McDonald 1983a, 1983b; McDonald et al. 1983; Wood 1988a). Under low calcium concentrations, as in the present study, chloride loss tends to equal or exceed sodium loss, thereby preventing acidic equivalent uptake.

Ammonia excretion as NH_4^+ is thought to be linked with Na^+ uptake (reviewed by Evans 1982). Wright and Wood (1985) showed that acute acid exposure reduced ammonia efflux by only 30% in rainbow trout but completely inhibited sodium influx, indicating that under acid conditions, NH_3 diffusion must account for the major part of ammonia excretion. At low pH, water becomes an almost infinite sink for NH_3 (by instantaneous interconversion to NH_4^+), so the P_{NH_3} gradient for NH_3 diffusion is greatly elevated. This effect probably contributed to the increase in ammonia excretion observed during our experiment and seen in several other studies under more severe exposure once the initial inhibition was attenuated (reviewed by Wright and Wood 1985). However, ammonia can only be excreted as fast as it is produced, so the long-term progressive increase in ammonia excretion must have had a metabolic basis. The cause is probably increased protein catabolism. Such an hypothesis is supported by our observation of a substantial increase of plasma cortisol which lasted throughout the 81 d of chronic acid exposure in rainbow trout (C. Audet and C. M. Wood, in prep.). It is surprising that in the first hours, no inhibition at all of ammonia efflux was observed in concordance with the immediate partial inhibition of Na^+ uptake. One possible explanation is that inhibited NH_4^+ efflux (via Na^+/NH_4^+ exchange) was stoichiometrically replaced by increased NH_3 efflux (due to the improved diffusion gradient). An alternate explanation is that Na^+/Na^+ exchange was inhibited more by pH = 4.8 than was Na^+/NH_4^+ exchange, which would support the argument about exchange diffusion presented earlier. Again, more data are needed to resolve this point.

The plasma ions and osmolality data followed the trend expected from the flux measurements. There were substantial decreases in plasma Na^+ and Cl^- during the period of negative net fluxes, and the timing of their stabilization agreed extremely well with the recovery of J_{net}. The larger decrease of plasma Cl^- over plasma Na^+ was in accord with more negative net fluxes of Cl^- than Na^+ in the first weeks of pH 4.8 exposure. As plasma osmolality stabilized fairly rapidly compared with plasma ions, with lesser absolute and relative decreases, other parameters must be involved in the maintenance of stable plasma osmotic pressure. Plasma glucose is certainly one of them. Impaired iono-osmoregulation was also found in rainbow trout (Giles et al. 1984) and Arctic char (*Salvelinus alpinus*) (Jones et al. 1987) following 22 and 14 d of sublethal acid exposure in hard water. Other studies pointed to similar iono-regulatory disturbances on a long-term basis during sublethal exposure of various durations in soft water: brook trout, pH 4.6, 1 yr (Muniz and Leivestad, as reported by Leivestad et al. 1976); juvenile Atlantic salmon (*Salmo salar*), pH 4.2–4.7, 4 mo (Saunders et al. 1983); white sucker (*Catostomus commersoni*) and pumpkinseed (*Lepomis gibbosus*), pH < 5.0, 19 d (Fraser and Harvey 1984); Atlantic salmon, white sucker, and alewife (*Alosa pseudoharengus*), pH 4.9–5.2, fish captured in the wild (Lacroix 1985); juvenile Atlantic salmon, pH 4.8–5.6, 50 d, field conditions (Lacroix and Townsend 1987). In contrast, Wendelaar Bonga et al. (1984a) showed recovery of plasma osmolality and almost complete restoration of plasma Na^+ in tilapia after 40 d of exposure to pH 4 or 5 in hard water. McWilliams (1980) also showed a recovery of plasma ions after 40 d of exposure of brown trout to very mild acid conditions (pH 6.0) in hard water. Wood et al. (1988a, 1988b, 1988c) showed no significant changes in plasma electrolytes in brook trout (*Salvelinus fontinalis*) kept for 10 wk at pH = 5.2 or 10 d at pH 4.8 in soft water. Thus, there appear to exist considerable species differences in the ability to adapt to sublethal acid exposure.

No significant decrease of plasma K^+ over time was observed, while the overall mean K^+ net flux was negative. The drop in muscle K^+ without simultaneous changes in muscle water points to an intracellular source for this loss. In a recent field study on long-term sublethal acid exposure in juvenile Atlantic salmon, Lacroix and Townsend (1987) similarly found no changes in plasma K^+ levels. However, plasma K^+ tended to increase through loss from the intracellular fluid in several previous studies on acute acid stress (McDonald and Wood 1981; Hōbe et al. 1984; Stuart and Morris 1985).

No changes in Ca^{2+} fluxes or plasma Ca^{2+} were observed, but muscle Ca^{2+} was dramatically increased after 3 mo of sublethal acid exposure. The meaning of this observation is unclear, although it is known that acid exposure interferes with calcium homeostasis and causes a mobilization of Ca^{2+} from bone in tilapia (Wendelaar Bonga et al. 1987). Previous studies employing more severe acid exposure (pH 4.0–4.5) reported responses ranging from no change in muscle Ca^{2+} (Neville 1979; Sadler and Lynam 1986) to significant increases (Hōbe 1987).

Increased plasma protein concentrations have been almost universally observed in previous studies on acute acid exposure and probably result from changes in plasma volume secondary to ionic disturbances, as discussed by Milligan and Wood (1982). A similar explanation probably applies to the chronic increase in plasma protein observed in the present study. Previous acute studies have also found an increase in hemoglobin levels comparable with that of the present investigation, again resulting from plasma volume contraction. However, the chronic increase in MCHC index is exactly opposite to that seen in any previous acute study. MCHC usually decreases greatly, the expected stress response indicative of cell swelling (Soivio and Nikinmaa 1981) and ionic dilution (Milligan and Wood 1982). The apparent shrinkage during chronic sublethal exposure may be viewed as a mechanism to prevent viscosity increases associated with hemoconcentration; however, its cause is unknown. This effect was not seen during chronic acid exposure of brook trout to pH = 5.2 in soft water (Wood et al. 1988a), but McWilliams (1980) found a decrease in hematocrit

(MCHC not measured) in brown trout chronically exposed to pH = 6.0 in hard water.

The chronic increase in blood glucose level agrees with previous studies in both hard water (Brown et al. 1984; Jones et al. 1987; Scherer et al. 1986; Tam et al. 1987) and soft water (Wood et al. 1988c). Both this plasma glucose elevation and the increased ammonia excretion were probably associated with the high plasma cortisol levels observed during the present study (C. Audet and C. M. Wood, in prep.). High cortisol levels are generally associated with glucose mobilization, stimulation of gluconeogenesis, and protein catabolism (Leach and Taylor 1980, 1982). These data indicate a maintained high level of stress in our fish chronically exposed to low water pH.

In summary, the general conclusion of this study is that rainbow trout are physiologically affected in a deleterious manner by chronic sublethal acid exposure. They undergo large changes in their internal physiological status, most of which are associated with disturbances in ionic exchange with the environment. However, the fish stabilize their internal condition after a few weeks of exposure, reaching a new physiological equilibrium without exhibiting any recovery back towards control levels. What is the biological meaning of such a new steady state? The following paper (Audet and Wood 1988) assesses whether this condition represents true physiological acclimation, in the sense of increased resistance to a more severe exposure.

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Do Rainbow Trout (*Salmo gairdneri*) Acclimate to Low pH?

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Audet, C., and C. M. Wood. 1988. Do rainbow trout (*Salmo gairdneri*) acclimate to low pH? Can. J. Fish. Aquat. Sci. 45: 1399–1405.

Adult rainbow trout (*Salmo gairdneri*) previously exposed to long-term sublethal acid stress (3 mo, pH 4.8) in artificial soft water (Ca^{2+} = 50, Na^+ = 50, Cl^- = 100 μeq·L^{-1}) were challenged with acute severe acid exposure (4.5–5 h, pH 4.0). Their response in terms of whole-body ionic exchanges and blood chemistry was compared with that of trout that had no previous history of acid exposure (naive fish). Acute pH 4.0 exposure caused significant ionoregulatory disturbances in both acid-preexposed and naive fish. Rates of net Na^+ and Cl^- body losses were twice as large in acid-preexposed fish as in naive fish. The two groups showed similar slight net uptake of acidic equivalents. However, the dynamics of acid–base exchange differed, especially with regard to ammonia excretion which was elevated in acid-preexposed fish and inhibited in naive fish. A larger decrease in plasma Na^+, red blood cell swelling, and the maintenance of high-stress indicators (elevated plasma glucose and ammonia excretion, depressed osmolality) confirmed that osmo-ionoregulatory disturbances were more intense in acid-preexposed fish than in naive fish. Thus, long-term sublethal acid exposure did not improve but rather significantly decreased the ability of rainbow trout to respond to more severe acid stress. We conclude that acclimation to acid stress does not occur in rainbow trout.

Des truites arc-en-ciel adultes (*Salmo gairdneri*), préalablement exposées à un stress acide sous-létal de longue durée (3 mo, pH 4,8) en eau douce à faible teneur ionique (Ca^{+2} = 50, Na^+ = 50, Cl^- = 100 μeq·L^{-1}), ont été exposées pour quelques heures à un pH acide plus sévère (stress acide aigü, 4,5–5 h, pH 4,0). Leur réponse, en termes d'échanges ioniques corporels et de paramètres sanguins, a été comparée à celle de truites n'ayant jamais connu de conditions acides. Une exposition aigüe à pH 4,0 a causé une perturbation des mécanismes iono-régulateurs chez les poissons pré-exposés ainsi que chez ceux non pré-exposés à l'acidité. Les taux de pertes nettes de Na^+ et de Cl^- ont été deux fois plus élevés chez les poissons pré-exposés à l'acidité. Une légère entrée nette d'équivalents acide a été observée chez les deux groupes mais la dynamique des échanges acide-base s'est avérée être différente pour chacun d'eux. Ainsi l'excrétion d'ammoniaque a été accrue chez les truites pré-exposées à l'acidité alors qu'elle était inhibée chez celles non pré-exposées. La chute plus prononcée de Na^+ plasmatique, le gonflement des globules rouges et le maintien d'un taux élevé d'indicateurs de stress (glucose plasmatique et excrétion d'ammoniaque élevés, osmolalité abaissée) ont confirmé que les truites pré-exposées à l'acidité connaissaient des perturbations plus importantes au niveau osmo-iono-régulation que celles non pré-exposées. L'exposition à un stress acide sous-létal prolongé n'a donc pas amélioré, mais décru, la capacité de la truite arc-en-ciel à répondre à un stress acide plus sévère. Nous concluons qu'il n'y a pas eu d'acclimatation à l'acidité chez la truite arc-en-ciel.

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Salmonids collected from naturally acidified soft water show greater physiological resistance to acid challenge in the laboratory (McWilliams 1980a, 1982, 1983; Brown 1981), but it is unclear whether this results from acclimation, or simply from natural selection, for acid tolerance is a heritable trait (Leivestad et al. 1976; Swarts et al. 1978). In general, the results of laboratory tests on acclimation have been negative or equivocal, but few have been performed under environmentally realistic water chemistry conditions (discussed in McWilliams 1980a, 1980b; Wood 1988).

In the accompanying paper (Audet et al. 1988), we have described the physiological effects on rainbow trout (*Salmo gairdneri*) of long-term sublethal acid exposure (3 mo at pH 4.8) in artificial soft water (ASW) closely duplicating water chemistry in the field. Following severe initial disturbances in ionoregulatory and related functions, most ionoregulatory parameters stabilized after the first few weeks at a new steady state. However, there was no indication of recovery back to control levels. The goal of the present study was to test whether this preexposure conferred increased tolerance when the fish were challenged with more severe acidity, which would constitute evidence for true acclimation. To test this hypothesis, we compared the response of ASW-acclimated rainbow trout held in circumneutral pH conditions (pH 6.5; naive fish) with trout exposed to long-term sublethal acid conditions (3 mo, pH 4.8, acid-preexposed fish) when both groups were submitted to acute, potentially lethal acid conditions (4.5–5 h, pH 4.0). These experimental conditions may simulate field conditions in some acidified streams where fish are chronically exposed to sublethal pH levels, with sudden brief pulses to acute lethal levels due to snowmelt or rainstorm runoff. Whole-body ion fluxes and related physiological parameters were examined to

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assess whether either the nature or the magnitude of responses to acute acid stress was different.

Methods

This study was performed concurrently with that of the accompanying paper (Audet et al. 1988) which provides complete information on fish holding, experimental protocols, and analytical methodology (unidirectional fluxes of Na^+ and Cl^-, net fluxes of Na^+, Cl^-, K^+, Ca^{2+}, ammonia, titratable acidity, acidic equivalents, plasma levels of Na^+, Cl^-, K^+, Ca^{2+}, glucose, osmolality, blood concentrations of hemoglobin, hematocrit, mean cell hemoglobin concentration (MCHC), and epaxial muscle water content). All fish were acclimated to ASW ($[Na^+]$ and $[Ca^{2+}] = 50$, $[Cl^-] = 100$ $\mu eq \cdot L^{-1}$) for 2–3 mo (pH 6.5 ± 0.3) prior to experiment. The temperature and photoperiod were kept constant at 15 ± 1°C and 24 h of light, respectively.

In the experiments reported here, two groups of adult rainbow trout (200–300 g) of both sexes were submitted to acute severe acid stress: 4.5–5 h in pH 4.0 (±0.1) ASW, acidified with H_2SO_4. Such exposure is invariably lethal within 0.5–3 d in previously unexposed trout. One group had been previously exposed to long-term sublethal acid conditions (3 mo at pH 4.8, as described by Audet et al. 1988) and were called "acid-preexposed fish." The other group was kept under circumneutral conditions (pH ≈ 6.5) prior to challenge with acute lethal acid exposure and were called "naive fish." Suitable control data prior to challenge for each group (i.e. acid-preexposed fish at pH 4.8; naive fish at pH 6.5) were furnished by the experiments described in Audet et al. (1988).

Fluxes were measured every hour from water samples taken during 4 consecutive h and final calculations based on the average of the four measurements. The total exposure time to pH 4.0 was close to 5 h taking into account the time to set up stable pH 4.0 conditions in the flux boxes, the addition of radioisotopes, and the 0.5 h left for the mixing of radioisotopes (see Audet et al. 1988). The fish were then individually anaesthetized and a terminal blood sample drawn by caudal puncture.

In additional sampling experiments (blood and tissue chemistry), individually labelled fish were transferred at time 0 to a 450-L tank, similar to the holding tank but supplied with ASW at pH 4.0. The fish were sampled after 4.5–5 h at pH 4.0. No significant differences within treatment groups were found for blood parameters taken from fish exposed in this manner versus those exposed in the flux boxes (t-test, $p \leq 0.05$), so the data were pooled. All samples were taken at times between 1330 and 1700. As in Audet et al. (1988), all the experiments were not done at the same time but controls were always run for each experimental group.

Statistics

The comparisons of control data between the two groups (i.e. acid-preexposed fish at pH 4.8; naive fish at pH 6.5) were drawn from Audet et al. (1988) (ANOVA, $p \leq 0.05$, followed by Tukey–Kramer test of comparison of means, $\alpha = 0.05$ (Sokal and Rohlf 1981)). All other statistical comparisons were done by t-test ($p \leq 0.05$). Data were tested for normality and for homogeneity of variances (Kolmogorov–Smirnov test and F_{max} test, respectively). All the data were normally distributed but some groups showed heterogeneous variances. In such cases, the t-test was then used with appropriate modifications (Sokal and Rohlf 1981).

Results

Ion Exchanges

Prior to acute acid exposure, net flux rates of the two major electrolytes, Na^+ and Cl^-, were similar in the two groups, although both J_{in} and J_{out} were significantly lower in the acid-preexposed fish (Fig. 1a, 1b). Whether or not the fish had been preexposed to sublethal acidity, acute exposure to pH 4.0 resulted in large net losses of Na^+ and Cl^- (Fig. 1a, 1b), consequent to an almost total inhibition of influx (J_{in}) and a tremendous increase of efflux (J_{out}). However, in acid-preexposed fish, the increase of J_{out} was almost twice that of the naive fish. This resulted in significantly larger net body Na^+ and Cl^- losses in the former. Net body K^+ losses also increased greatly during acute exposure in both acid-preexposed and naive fish (Fig. 1c); the increase was greater in the former, but the difference was not significant. We did not observe any significant disturbances of net Ca^{2+} flux in either group, the overall mean of net Ca^{2+} flux after 5 h at pH 4.0 being -11.1 ± 6.9 $\mu eq \cdot kg^{-1} \cdot h^{-1}$ ($N = 22$).

Similar net acidic equivalent uptake was observed in the two groups of fish prior to acute acid exposure (Fig. 1d). However, the titratable acidity and ammonia exchanges underlying these net acidic equivalent fluxes were very different in acid-preexposed and naive fish. Thus the elevated ammonia and titratable acidity fluxes caused by the pH 4.8 exposure persisted when acid-preexposed fish were submitted to pH 4.0. In contrast, this exposure resulted in a significant decrease of ammonia and no change in titratable acidity flux in naive fish. As a result, net acidic equivalent uptake increased in the naive fish during the pH 4.0 exposure whereas no changes occurred in the dynamics of acidic equivalent exchange in the acid-preexposed fish.

Blood and Tissue Chemistry

Control levels of plasma Na^+ and Cl^- were substantially lower in the acid-preexposed fish (Fig. 2a, 2b). A further drop in plasma Na^+ was observed following acute lethal acid exposure in acid-preexposed fish but not in naive fish (Fig. 2a). In acid-preexposed fish, the decrease in the plasma Na^+ concentration attributable to the pH 4.0 exposure was three times greater than the one observed in naive fish. Plasma Cl^- concentration decreased by approximately the same magnitude in the two groups (Fig. 2b). A slight increase in plasma Ca^{2+} (Fig. 2c) and a slight decrease in plasma K^+ (Fig. 2d) were observed in naive fish, while no changes occurred in acid-preexposed fish. Nevertheless, acid-preexposed and naive fish presented similar Ca^{2+} and K^+ plasma concentrations after 5 h of pH 4.0 exposure.

Prior to acute acid exposure, plasma osmolality (Fig. 3a) was significantly lower and plasma glucose (Fig. 3b) and protein levels (Fig. 3c) higher in the acid-preexposed trout. Despite the greater loss of plasma electrolytes (mainly Na^+; Fig. 2a) at pH 4.0 in the acid-preexposed group, there was no significant change in plasma osmolality in these fish, while in naive fish, plasma osmolality decreased (Fig. 3a). The response may have been obscured by greater variability in the preexposed group. Plasma glucose and plasma protein concentrations increased in naive fish in contrast with acid-preexposed fish for which no such effects were observed (Fig. 3b, 3c). However, plasma glucose and protein concentrations were already elevated to significantly higher levels as a result of acid preex-

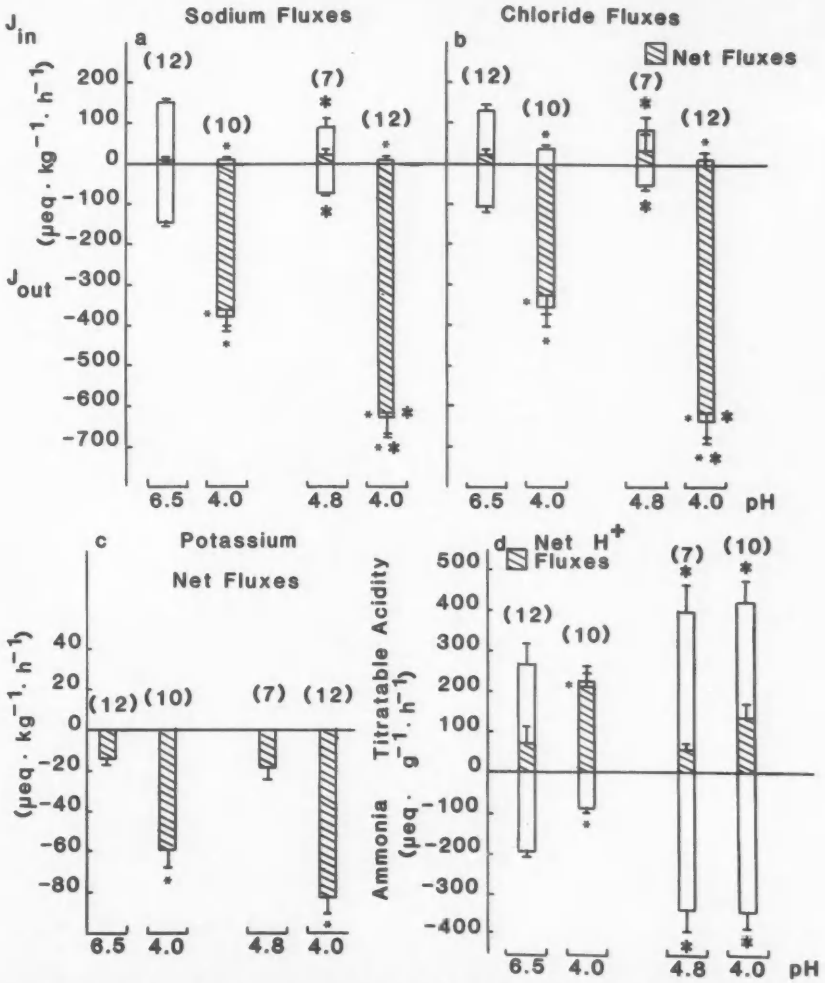

FIG. 1. Effects of acute pH 4.0 exposure on whole-body ion fluxes in naive and acid-preexposed rainbow trout. (a) Unidirectional and net sodium fluxes; (b) unidirectional and net chloride fluxes; (c) net potassium fluxes; (d) net fluxes of acidic equivalents, ammonia, and titratable acidity. Within each panel, from left to right the columns represent sodium fluxes in naive fish tested at pH 6.5 (column 1), in naive fish challenged with pH 4.0 (column 2), in fish after 3 mo of pH 4.8 exposure (column 3), and in fish preexposed for 3 mo to pH 4.8 and challenged with pH 4.0 (column 4). The small asterisks represent significant effects ($p \leq 0.05$) of pH 4.0 exposure in naive fish (column 1 vs. column 2) or in acid-preexposed fish (column 3 vs. column 4). The large asterisks represent significant effects ($p \leq 0.05$) of long-term sublethal acid exposure (column 1 vs. column 3) or significant differences ($p \leq 0.05$) between the two groups challenged with pH 4.0 (column 2 vs. column 4). Numbers in parentheses represent the number of fish in each group.

posure. No significant changes in water content in epaxial white muscle, as a result of pH 4.0 exposure, were detected in either acid-preexposed or naive fish (Fig. 3d). However, the combined effects of long-term sublethal and acute lethal exposure significantly increased muscle water content relative to that of naive fish exposed to pH 4.0 conditions.

Acute pH 4.0 exposure did not further affect the already elevated hemoglobin concentration in acid-preexposed fish, while hemoglobin concentration doubled in naive fish (Fig. 4a). There was also a significant 29% increase of the hematocrit in naive fish, while a similar (22%) but not significant trend was observed in acid-preexposed fish. Nevertheless, final hematocrit level after pH 4.0 exposure was not different between naive and acid-preexposed fish. The overall effect of these changes in hemoglobin concentration and hematocrit level was a 15% decrease of the MCHC after acute pH 4.0 exposure in acid-preexposed fish compared with a 60% increase in naive fish (Fig. 4c).

Discussion

The present study indicated that long-term sublethal acid exposure did not improve the physiological ability of softwater-

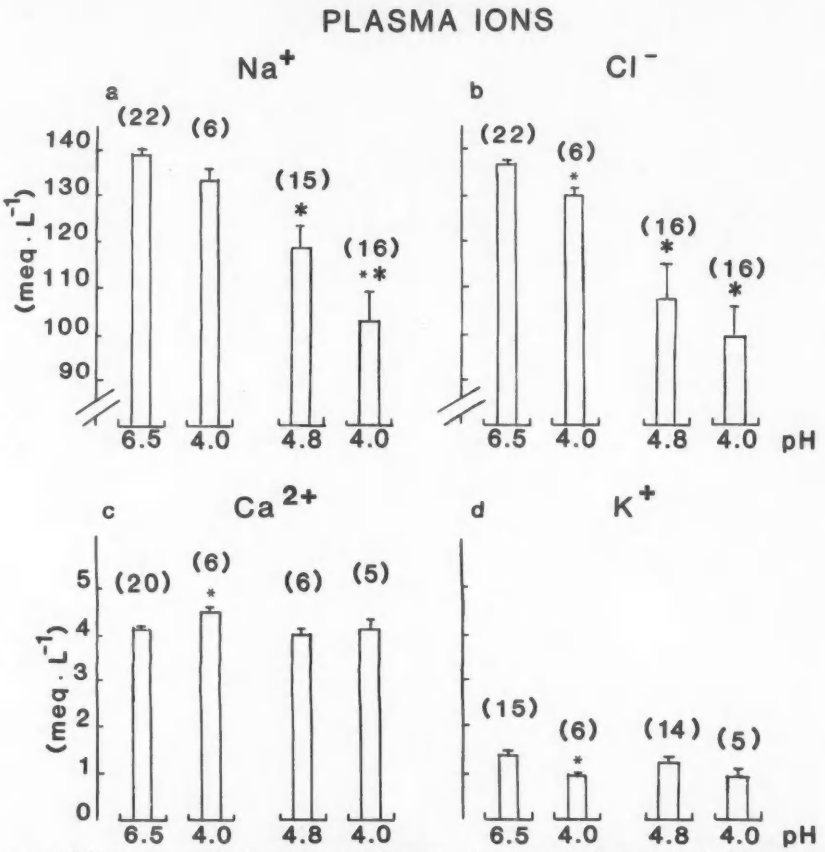

FIG. 2. Effects of acute pH 4.0 exposure on plasma ions in naive and acid-preexposed rainbow trout. (a) Plasma Na^+; (b) plasma Cl^-; (c) plasma Ca^{2+}; (d) plasma K^+. See legend to Fig. 1 for other details.

acclimated rainbow trout to respond to acute lethal acid exposure. For both groups, we observed large negative net fluxes of Na^+ and Cl^- resulting from inhibition of J_{in} and stimulation of J_{out}. Such observations are in agreement with earlier studies on lethal acid stress in softwater-acclimated rainbow trout (McDonald 1983b; McDonald et al. 1983; Hōbe et al. 1984). However, the interesting and novel observation is that acid-preexposed fish lost their body ions twice as fast as naive fish, essentially due to a greater stimulation of J_{out}. This occurred despite the fact that plasma Na^+ and Cl^- levels were lower, and therefore the driving diffusive gradients from blood to water were reduced.

Thus, preexposure clearly rendered the fish more, rather than less, sensitive to a more severe challenge. Acclimation did not occur.

The stimulation of J_{out} in fish submitted to acid stress is thought to result from an increase in gill membrane permeability following leaching of Ca^{2+} away from the paracellular channels (McWilliams 1983; McDonald 1983a; Marshall 1985). Prolactin is thought to oppose this effect and reduce gill membrane permeability to water and ions (reviewed by Nicoll 1981). In the accompanying paper (Audet et al. 1988), we proposed that chronic prolactin mobilization might explain the maintained reductions in J_{out}^{Na+} and J_{out}^{Cl-} seen during 3 mo of exposure to pH 4.8. In tilapia, Wendelaar Bonga et al. (1984, 1987) reported massive degranulation of prolactin cells during the first few hours following exposure to pH 4.0. If the long-term sublethal exposure in some way decreased the ability of the fish to either mobilize further prolactin (e.g. through exhaustion of stores or synthetic capacity) or respond to prolactin (e.g. desensitization of receptors during acute exposure), then this might explain the greater Na^+ and Cl^- effluxes in the preexposed fish. Alternatively, the chronic response may have damaged some other mechanisms (e.g. mucus secretion) involved in resistance to the "permeabilizing" effect of acute, severe acid exposure. Undoubtedly, more work is needed to resolve this question.

The other physiological disturbances resulting from acute exposure to pH 4.0 can be viewed as direct consequences of the massive rates of ion loss from the gills. Indeed, the measured changes in plasma ions were very close to those predicted by the measured flux rates and previous estimates of internal Na^+ and Cl^- pools in trout (Wood and McDonald 1982; McDonald 1983b). The effects of such rapid ion losses have been discussed in detail previously (McDonald 1983b; Wood and McDonald 1982; Wood 1988), so will not be dealt with here. However, there were two important qualitative differences between the responses of naive fish and acid-preexposed fish which deserve comment.

Firstly, in naive fish, acute exposure to pH 4.0 caused a 50% drop in ammonia excretion whereas in acid-preexposed fish,

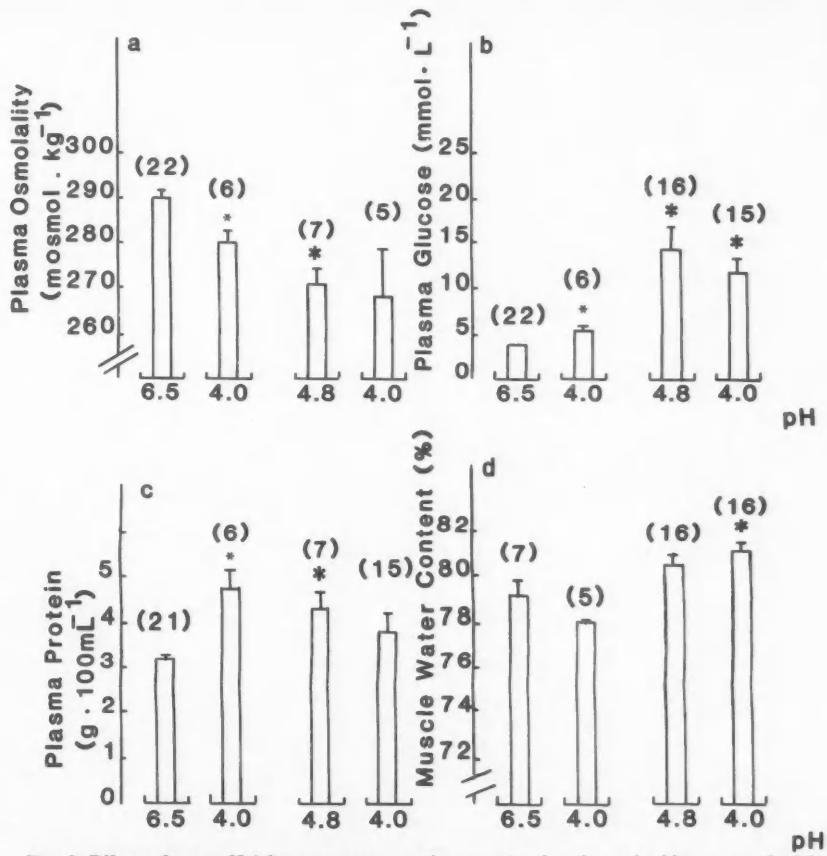

FIG. 3. Effects of acute pH 4.0 exposure on osmotic parameters in naive and acid-preexposed rainbow trout. (a) Plasma osmolality; (b) plasma glucose; (c) plasma protein; (d) water content in epaxial white muscle. See legend to Fig. 1 for other details.

ammonia efflux remained at chronically elevated levels. Wright and Wood (1985) found a similar drop in ammonia excretion of naive fish exposed to pH 4.0 in hard water and related it to an inhibition of J_{in}^{Na+}, i.e. a blockade of Na^+/NH_4^+ exchange. A similar explanation is resonable for the naive fish of the present study because the drop in ammonia efflux was apparently equimolar in the inhibition of J_{in}^{Na+} ($\approx$ 130 $\mu eq \cdot kg^{-1} \cdot h^{-1}$). In the acid-preexposed trout, J_{in}^{Na+} was already partially inhibited by chronic exposure, and ammonia production rates were chronically elevated (Audet et al. 1988). These fish probably relied on NH_3 diffusion to a greater extent; any inhibition of the small remaining Na^+/NH_4^+ component would have been masked by the increased NH_3 efflux along the improved diffusion gradient at pH 4.0.

Secondly, acute exposure of acid-preexposed fish to pH 4.0 did not cause the classic hemoconcentration evidenced by increased hemoglobin and plasma protein concentrations in the naive fish (see Milligan and Wood 1982 for a full discussion of this response), although the red blood cells swelled, as evidenced by a fall in MCHC. The explanation is unclear, but it should be noted that the pre-exposed fish already manifested chronic increases in hemoglobin and plasma protein. The capacity for further response may have been limited.

Few previous studies have compared the responses to more severe acid stress between fish preexposed to acid conditions and naive fish. Falk and Dunson (1976) compared survival time to pH 3.1–3.5 in brook trout (*Salvelinus fontinalis*) but did not find that preexposure to sublethal acidity improved the response of fish. Studies done on the same species by Wood et al. (1988a, 1988b) showed physiological evidence of acclimation to combined acid *plus* aluminium stress after preexposure (10 wk) to sublethal pH (5.2) and aluminum (150 $\mu g \cdot L^{-1}$). However, there was no evidence that preexposure to sublethal pH (5.2) alone conferred greater tolerance, i.e. the acclimation appeared to be to aluminium and not to acidity. These results are therefore in general accord with those of the present study.

In conclusion, acclimation did not occur. No improvements of the ability to respond to acute lethal acid stress were observed in rainbow trout chronically preexposed to sublethal acid stress in soft water. Moreover, larger rates of Na^+ and Cl^- efflux, faster decreases in plasma Na^+, and greater red blood cell swelling showed that they were in fact more sensitive to acute lethal acid stress than naive fish. These results, in association with maintained low osmolality and high levels of glucose and ammonia excretion (stress indicators), indicated that these fish were in poor physiological condition. The death of fish previously exposed to pH 4.8 is therefore expected to occur faster under lethal acid exposure. In the field, we predict that fish chronically exposed to pure acid stress in soft water will be more sensitive to episodic acid surges (e.g. rainstorm and snow-

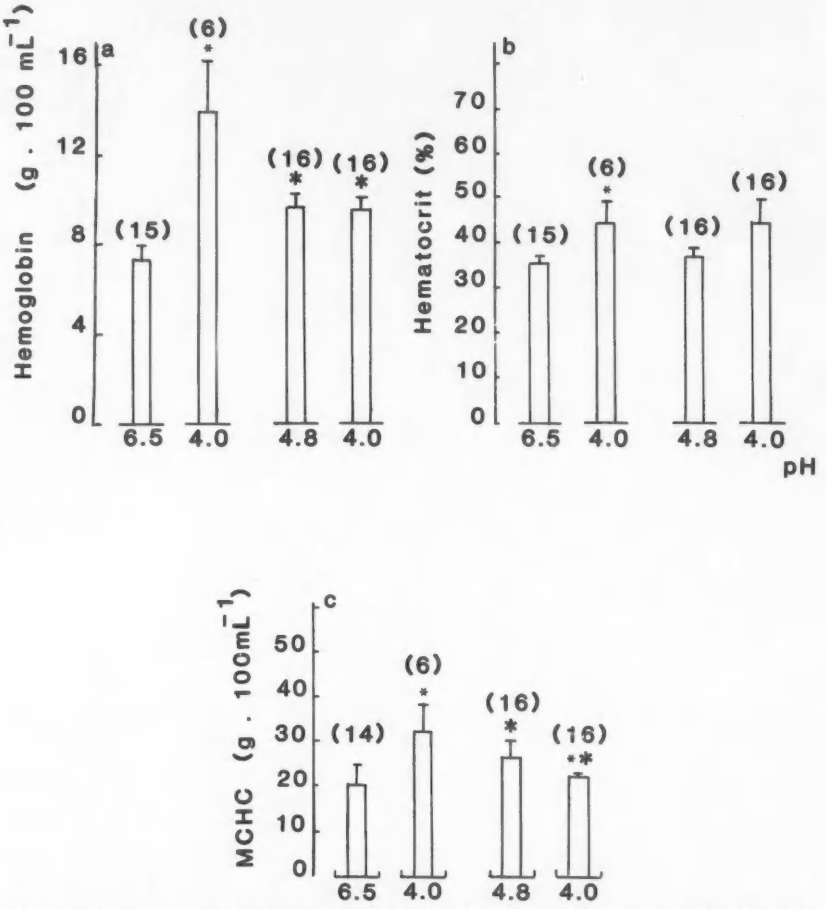

FIG. 4. Effects of acute pH 4.0 exposure on hematology in naive and acid-preexposed rainbow trout. (a) Blood hemoglobin concentration; (b) hematocrit level; (c) MCHC. See legend to Fig. 1 for other details.

melt runoff) than predicted by laboratory trials with naive fish. However, this interpretation may not apply when a genetic basis for acid tolerance has been favoured by natural selection, or when metals are simultaneously involved.

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Assessment of Sea Lamprey (*Petromyzon marinus*) Predation by Recovery of Dead Lake Trout (*Salvelinus namaycush*) from Lake Ontario, 1982–85[1]

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During 1982–85, 89 dead lake trout (*Salvelinus namaycush*) were recovered with bottom trawls in U.S. waters of Lake Ontario: 28 incidentally during four annual fish-stock assessment surveys and 61 during fall surveys for dead fish. During the assessment surveys, no dead lake trout were recovered in April–June, one was recovered in August, and 27 were recovered in October or November, implying that most mortality from causes other than fishing occurred in the fall. The estimated numbers of dead lake trout between the 30- and 100-m depth contours in U.S. waters ranged from 16 000 (0.08 carcass/ha) in 1983 to 94 000 (0.46 carcass/ha) in 1982. Of 76 carcasses fresh enough to enable recognition of sea lamprey (*Petromyzon marinus*) wounds, 75 bore fresh wounds. Assuming that sea lamprey wounding rates on dead fish were the same as on live ones of the same length range (430–740 mm), the probability of 75 of the 76 dead lake trout bearing sea lamprey wounds was 3.5×10^{-63} if death was independent of sea lamprey attack, thus strongly implicating sea lampreys as the primary cause of death of fish in the sample. The recovery of only one unwounded dead lake trout also suggested that natural mortality from causes other than sea lamprey attacks is negligible.

De 1982 à 1985, 89 carcasses de touladi (*Salvelinus namaycush*) ont été remontées des eaux américaines du lac Ontario suite à des chalutages de fonds. De ce nombre, 28 carcasses ont été récupérées par accident, au cours de quatre relevés annuels d'évaluation des stocks, et 61 carcasses au cours de relevés d'automne des poissons morts. Au cours des relevés d'évaluation, aucun touladi n'a été obtenu d'avril à juin, un l'a été en août et 27 en octobre ou novembre; ce qui porte à croire que la plus grande partie de la mortalité non causée par la pêche se produit en automne. Le nombre estimé de touladis morts se trouvant sur les fonds américains situés entre 30 et 100 m de profondeur était de 16 000 (0,08 carcasse/ha) en 1983 et de 94 000 (0,46 carcasses/ha) en 1982. Des 76 carcasses dont l'état permettait de reconnaître les blessures faites par la lamproie marine (*Petromyzon marinus*), 75 présentaient des blessures récentes. Si l'on suppose que le taux d'attaque par les lamproies noté chez les poissons morts était le même que celui noté chez les poissons vivants de la même gamme de longueurs (430–740 mm), la probabilité que 75 des 76 touladis morts présentant des blessures de lamproies soient morts de causes autres que l'attaque par les lamproies est de $3,5 \times 10^{-63}$, ce qui indique très fortement que les lamproies sont la principale cause de la mort des poissons de l'échantillon. Le fait qu'une seule des carcasses de touladi ne présente pas de blessures faites par les lamproies porte aussi à croire que la mortalité par autres causes est négligeable.

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The sea lamprey (*Petromyzon marinus*) is generally regarded as having caused, or at least contributed substantially to, the decline of the lake trout (*Salvelinus namaycush*) in the Great Lakes (Smith 1968). In an effort to return stocks to their former productivity, the Great Lakes Fishery Commission has directed a program to reduce sea lamprey populations since 1955. This program has greatly reduced the number of sea lampreys in most areas of the Great Lakes, primarily by the use of a selective lamprey larvicide (Pearce et al. 1980; Smith and Tibbles 1980). Since 1981 the Commission has advocated an integrated pest management approach to sea lamprey control (Great Lakes Fishery Commission 1981). This approach requires the measurement of the impact of sea lampreys on affected fish stocks (Sawyer 1980).

No direct field measurements of the numbers of fish killed by sea lampreys have been made. Instead, the effects of sea lampreys on their prey populations have been "inferred from correlations among (1) lamprey abundance and prey abundance and size distribution; (2) lamprey abundance and total prey mor-

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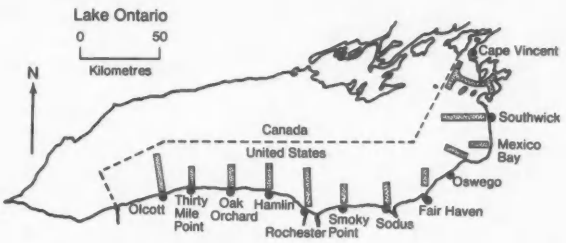

FIG. 1. Location of 12 Lake Ontario areas sampled with bottom trawls (from O'Gorman and Schneider 1986).

tality rates estimated by tagging and catch curves; and (3) lamprey wounding/scarring rates and total prey mortality rates" (Walters et al. 1980). Indirect measures have been used because only survivors of sea lamprey attacks were readily available for examination; those that died sank to the bottom and were seldom seen (Pycha 1980; Spangler et al. 1980). The general perception of a low density of dead fish and the enormous area of the Great Lakes have discouraged attempts to measure sea lamprey induced mortality by directly sampling the victims.

While conducting annual assessments of Lake Ontario's fish stocks in 1981, we observed enough dead lake trout (apparently killed by sea lampreys) in bottom-trawl catches to suggest the possibility of making inferences from such incidental recoveries. Consequently, during 1982–85 we recorded data, including those relating to sea lamprey wounding, on all dead lake trout recovered. We had three objectives: (1) to examine the apparent seasonal timing of natural mortality of lake trout based on our recoveries of dead lake trout; (2) to make provisional estimates of the minimum numbers of dead lake trout on the lake bottom; and (3) to examine the probable contribution of sea lamprey attacks to that mortality.

Methods

Recoveries of dead lake trout were made during two types of bottom-trawl tows. Tows during fish–stock assessment surveys during 1982–85 were made along the contour and usually lasted 10 min (a few lasted less than 10 min). Tows made solely to recover dead lake trout during 1983–85 were generally made across the contour and varied more widely in duration (up to 100 min). The longer tows enabled us to sweep a greater bottom area per day because relatively less time was spent setting and retrieving the gear. These two types of tows are hereinafter referred to as 10-min and cross-contour tows.

Two types of bottom trawls were used. A yankee trawl having a 12-m headrope was used for all 10-min tows during 1982–85 and for all cross-contour tows during 1983–84. A four-seam shrimp trawl having an 18-m headrope was used for most of the cross-contour tows in 1985, although some tows were made with the yankee trawl. Measured net spreads were 6.4 m for the yankee trawl and 14 m for the shrimp trawl. During 10-min tows, the yankee trawl was fitted with a cod end of 9-mm mesh (stretched measure). During cross-contour tows, both the yankee and shrimp trawls were fitted with cod ends of 89-mm mesh (stretched measure) to allow the escape of small fishes. The yankee trawl was towed by both the R/V *Kaho* of the U.S. Fish and Wildlife Service and the R/V *Seth Green* of the New York Department of Environmental Conservation. The shrimp trawl was towed only by the R/V *Seth Green*.

The 10-min tows were made during four major fish-stock assessment surveys each year during 1982–85. In 10 min the yankee trawl swept 0.5 ha. The range in hectares swept and the approximate dates for these four surveys during 1982–85 were 33.9–47.3 ha in late April – early May, 24.0–33.9 ha in early June, 33.3–44.7 ha in late July – early August, and 3.0–31.3 ha in late October – early November. Series of trawl tows were generally made at each of 12 locations during each survey (Fig. 1), with the following exceptions: no sampling was conducted in the Fair Haven area during June or July–August in any year or in the Cape Vincent area during October–November in any year, and sampling was conducted only in the Oswego area during October–November 1983 and only in the Olcott, Thirty Mile Point, Rochester, Smoky Point, Fair Haven, and Oswego areas during October–November 1984–85. Series of 10-min tows were also made in the Oswego area on 1 March 1983 and 15–16 February 1984. In general, tows were made at 10-m depth intervals throughout the depth range where the target species for that assessment was abundant, except during July–August, when tows were made at 5-m depth intervals. Because depth distributions of the target species differed, the range of depths fished varied among surveys: April–May, 8–150 m; June, 8–130 m; July–August, 15–75 m; and October–November, 25–150 m.

All cross-contour tows were restricted to October–November 1983–85; none were made in 1982. The tows were made from the 20- to 100-m contours in 1983 but were started at the 30-m contour in 1984–85 because gear damage had been excessive at depths less than 30 m. Because no dead lake trout were recovered in October–November in 10-min tows made at depths less than 40 m (Table 1), we believe that the 1983 cross-contour tows were comparable with those made in 1984–85. Such tows were made in all areas (Fig. 1) in 1983 but were limited mainly to the Olcott, Rochester, and Oswego areas in 1984 and 1985 because gear damage had been unacceptably high in the other areas. Cross-contour tows were also made between the 100- and 160-m depth contours in 1985.

All dead lake trout recovered in bottom trawls after 1981 were examined for sea lamprey wounds. Fresh wounds were defined as any type A lesions (King 1980) in stage I or II of healing, i.e. the skin at the attachment site was broken and there was no repigmentation of the descaled skin at the margins of the wound. During 1983–85, total lengths (millimetres) of all dead lake trout were recorded.

Because cross-contour tows were made only during fall, the seasonal distribution of mortality was described on the basis of catch and effort data from only the 10-min tows. Catch and effort were summed by fish-stock assessment survey during 1982–85. Effort was considered only for station depths of 15–130 m, the depth range of recovery for all dead lake trout in 10-min tows.

Estimates of the density and number of dead lake trout on the bottom in U.S. waters of Lake Ontario during October–November of each year during 1982–85 were made for two depth strata: 30–100 m and 100–160 m. Density (number per hectare) in each stratum was calculated as the total number of dead lake trout recovered in both 10-min and cross-contour tows divided by the total area (hectares) swept by the trawls. The total number of dead lake trout in each stratum was calculated by multiplying the estimated density (number per hectare) by the total area (hectares) in that stratum.

We estimated the proportion of dead fish bearing sea lamprey wounds by using recoveries from all sources. One dead lake

TABLE 1. Minutes towed and (in parentheses) catch of dead lake trout from bottom-trawl tows made during October–November 1982–85 and lasting 10 min. Expected catches were calculated from total catch and effort, assuming an even distribution with catch proportional to effort. Totals and expected catches were calculated only for locations where effort was similar in all three years.

	Depth (m)										
Year	25	35	45	55	65	75	85	95	110	130	150
1982	0(0)	30(0)	80(2)	80(4)	80(0)	90(4)	70(1)	70(1)	50(0)	20(1)	20(0)
1984	10(0)	20(0)	60(1)	60(1)	60(2)	60(0)	50(0)	50(1)	60(2)	30(0)	20(0)
1985	10(0)	10(0)	50(0)	50(0)	50(0)	50(1)	50(0)	50(0)	60(1)	50(1)	20(0)
Total	20(0)	60(0)	190(3)	190(5)	190(2)	200(5)	170(1)	170(2)	170(3)	100(2)	60(0)
Expected	0.3	0.9	2.9	2.9	2.9	3.0	2.6	2.6	2.6	1.5	0.9

	Location									
Year	Olcott	Thirty Mile Point	Oak Orchard	Hamlin	Rochester	Smoky Point	Sodus	Fair Haven	Oswego	Mexico Bay
1982	70(1)	70(1)	60(1)	60(1)	70(3)	10(0)	70(2)	30(1)	100(3)	50(0)
1984	70(1)	70(2)			80(1)	70(1)		30(0)	110(2)	50(0)
1985	70(1)	80(0)			80(0)	80(0)		30(2)	110(0)	
Total	210(3)	220(3)			230(4)			90(3)	320(5)	
Expected	3.5	3.7			3.9			1.5	5.4	

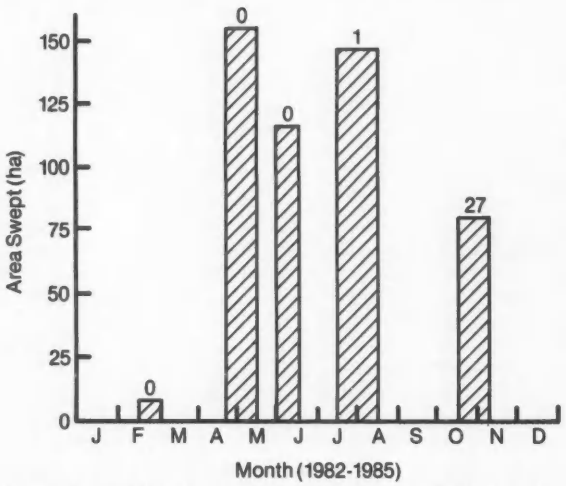

FIG. 2. Catch of dead lake trout and total bottom-trawl effort in Lake Ontario during five seasonal periods, 1982–85. Widths of the bars are inclusive dates and heights are total area swept. The number of dead lake trout recovered is given above each bar.

trout recovered with a bottom trawl in October 1984 by Brandt (1986) was included in that calculation.

Live lake trout were collected in gill nets set on the bottom during September of each year during 1982–85. In each year, nets were set at each of four depths at a randomly selected location in each of 17 subdivisions of U.S. waters. Each net contained mesh sizes of 51–152 mm (stretched measure) by 13-mm intervals. Total lengths and sea lamprey wounding data were recorded for lake trout captured. Wounds were classified according to the criteria of King (1980) and wounding rate was expressed as the mean number of stage I and II wounds per fish. Estimates of the number of lake trout harvested by anglers and the spatial distribution of the harvest were obtained from direct-contact creel censuses conducted on Lake Ontario in 1984 and 1985 by the New York Department of Environmental Conservation (Cape Vincent Fisheries Station, unpubl. reports).

Results and Discussion

Seasonal Distribution of Natural Mortality of Lake Trout

Nearly all incidental catches of dead lake trout in 10-min tows were made in October or November. Of 28 dead lake trout recovered, 27 were taken during those two months, even though fishing effort was least during this seasonal period (Fig. 2); one was recovered during July–August. The length range of the dead lake trout (430–740 mm) corresponded approximately to age 3+ and older (Cape Vincent Fisheries Station, unpubl. data).

The disproportionately high number of dead lake trout recovered during October and November (Fig. 2) indicated that most natural mortality of lake trout of age 3+ and older in Lake Ontario (including mortality from sea lamprey predation) that occurs between late March and early November occurs during fall. The limited sampling during winter in 1983 and 1984 also suggested low winter mortality, but too little sampling was done to support sound conclusions. Assuming that dead lake trout required several weeks to decompose beyond recognition, regardless of time of year, the lake trout that died during seasons other than fall and that died within the depth range sampled during each season should have been recovered in about as large a proportion as those in the fall, unless a smaller proportion of carcasses were within the range of sampling depths during March–August. Such a distribution seems unlikely because more live lake trout of age 3+ and older were caught in fish-stock assessments during April–August than during October–November (J. E. Elrod, Oswego Biological Station, pers. comm.). Furthermore, the median depth of capture for lake trout as reported by anglers to creel census agents in each month from April to August 1986 (Cape Vincent Fisheries Station, unpubl. data) was well within the range of depths trawled during the respective fish-stock assessment surveys.

Our conclusion that most natural mortality occurs during fall also assumes that lake trout dying of natural causes from March through November settle in equal proportions to the lake bottom. While we cannot prove that the fate of dead lake trout is not different between seasons, neither is there any evidence to suggest such a difference. No dead lake trout were recovered

from the lake surface during about 800 vessel days during 1982–85, despite awareness by the crews of the R/V *Kaho* and R/V *Seth Green* of the importance of recovering dead lake trout and despite that a much larger area can be scanned visually from the deck or pilot house of these vessels than can be swept with trawls.

Density and Population Estimates

Incidental catches of dead lake trout during fall appeared to follow the Poisson distribution. We compared catches made in 10-min tows during October–November surveys in 1982, 1984, and 1985, using a χ^2 goodness of fit test, with the Poisson. Only tows lasting the full 10 min were used. In 1982, when no cross-contour tows were made, the catch distribution was compared with a Poisson distribution fitted to its sample mean. To conserve degrees of freedom, we compared the 1984 and 1985 catch distributions with Poisson distributions fitted to means estimated independently from cross-contour tows made in those years. For all three years the fit was satisfactory (1982, $P > 0.25$; 1984, $P > 0.50$; and 1985, $P > 0.50$). Also, there were no depths or locations with catches distinctly higher or lower than the expected catches calculated assuming that catch was strictly proportional to effort (Table 1).

Because cross-contour tows varied in length, the catches could thus not be similarly tested, but were assumed to also follow a Poisson distribution. We therefore assumed a Poisson distribution in calculating confidence intervals for catch (number caught), density (catch per area swept), and number (density times stratum area).

The yankee trawl caught 1.7 times as many lake trout carcasses, per hectare swept, as the shrimp trawl. However, because the confidence intervals for estimated carcass density (assuming the Poisson distribution) overlapped even at the 0.5 level, we combined catch and effort for the two gears.

The numbers of dead lake trout on the bottom in U.S. waters of Lake Ontario each fall during 1982–85 appeared to be substantial. In the 30- to 100-m depth stratum, the estimated numbers of dead lake trout ranged from 16 000 (0.08 carcass/ha) in 1983 to 94 000 (0.46 carcass/ha) in 1982 (Table 2). The apparent densities in the 30- to 100-m and 100- to 160-m depth strata were fairly similar in 1982 and 1984, but the limited effort in the deeper stratum produced too few carcasses for population estimates (Table 2). Sampling was not conducted at depths greater than 100 m in 1983. In 1985, when there was substantial effort in both strata, the estimated number of dead lake trout in the combined strata was 2.6 times the estimate for the 30- to 100-m stratum alone (the range for this ratio, calculated by using the limits of the 95% confidence intervals from Table 2, was 1.3–4.9). Assuming a ratio of 2.6:1 in all years, the estimated numbers of dead lake trout between the 30- and 160-m depth contours in the fall of each year were 240 000 in 1982, 41 000 in 1983, 176 000 in 1984, and 51 000 in 1985.

We believe that our estimates of numbers of dead lake trout in U.S. waters of Lake Ontario are conservative, for several reasons. First, in our calculations we assumed that all dead lake trout were on bottom, an assumption that if not correct would result in underestimates; however, since we saw few dead lake trout on the surface, any errors related to this assumption were probably small. Second, we did not take into account any lake trout that died after the fall surveys or that died before the fall surveys and had decomposed beyond recognition, omissions that must have led to some degree of underestimation. And third, we assumed that gear efficiency was 100%, an almost certain overestimation resulting in underestimates of the number of dead lake trout.

There was also a potential bias in our estimates because sampling was restricted to smooth-bottomed areas, although the nature of that bias was less certain. We assumed that densities of dead lake trout in smooth-bottomed areas (where we could trawl) were representative of densities in all areas. There were no data with which to examine the validity of this assumption or on which to speculate whether overestimates or underestimates would be the more likely.

Sea Lamprey as a Mortality Factor

Nearly all dead lake trout that we recovered bore fresh sea lamprey wounds. For all effort combined during 1982–85, we recovered 89 dead fish that could be identified as lake trout. Of these, 76 were still in sufficiently early stages of decomposition to enable the recognition of sea lamprey wounds. Type A, stage I or II wounds were found on 75 (99%) of the fish. The 95% confidence interval for the proportion of dead lake trout bearing wounds was 0.95–1.00.

Sea lamprey attacks probably caused, either directly or indirectly, the death of nearly all the dead lake trout we examined. If the probability of death was independent of sea lamprey attack, the wounding rates should have been the same for both live and dead lake trout. However, live lake trout captured in

TABLE 2. Catches, estimated densities, and population estimates by depth stratum for dead lake trout in U.S. waters of Lake Ontario, based on recoveries made with bottom trawls during October–November 1982–85. Areas used to estimate number from density were 204 000 ha for the 30- to 100-m depth stratum and 241 000 ha for the 100- to 160-m depth stratum. CI = confidence interval; NC = not calculated.

Year	Depth stratum (m)	Catch	Area swept (ha)	Density		Population estimate	
				Number/ha	95% CI	Number	95% CI
1982	30–100	12	26.3	0.46	0.20–0.71	94 000	41 000 – 145 000
	100–160	1	4.5	0.22	0.00–0.66	NC	NC
1983	20–100	7	90.5	0.08	0.02–0.13	16 000	4 000 – 27 000
1984	30–100	16	47.1	0.34	0.17–0.51	69 000	35 000 – 104 000
	100–160	2	5.5	0.36	0.00–0.87	NC	NC
1985	30–100	36	366.5	0.10	0.07–0.13	20 000	14 000 – 27 000
	100–160	6	47.1	0.13	0.03–0.23	31 000	7 000 – 55 000

gill nets in U.S. waters of Lake Ontario in September during 1982–85 bore far fewer wounds than the dead lake trout we examined. Total lengths of dead lake trout ranged from 430 to 740 mm. The mean number of type A, stage I or II wounds per live lake trout 431–733 mm long averaged 0.087 during 1982–85 and ranged from 0.044 (n = 1313) in 1983 to 0.139 (n = 640) in 1982. If we made the conservative assumption that during 1982–85 the average proportion of live lake trout with wounds was 0.139, the estimated probability of observing 75 wounded fish in our sample of 76 was 3.5×10^{-63} (calculated from the binomial distribution).

The most obvious conclusion is that sea lamprey attack was the predominant cause of death for the fish in our sample. Such a disproportionate observation could also be explained if sea lampreys selectively attacked fish already dying of other causes or if the lamprey-killed fish were unique in settling to the bottom. However, selection of dying fish has not been reported for sea lampreys, except for fish held in gill nets (Christie and Kolenosky 1980), and there is no evidence to suggest a difference in the fate of the carcasses of lake trout dying from sea lamprey attacks and from other sources of natural mortality.

If the observed pulse of mortality in the fall was in fact caused by sea lamprey attacks, the timing would also agree well with previous studies of sea lamprey – fish interactions. Host mortality is known to increase with temperature and decrease as the host to lamprey weight ratio increases (Farmer 1980). In fall, sea lampreys approach their maximum size and the host to lamprey weight ratio approaches its minimum. From a bioenergetics model, using a temperature regime based on Lake Michigan data, Kitchell and Breck (1980) predicted that blood consumption by sea lampreys peaked in early November. Swink and Hanson (1986) showed that lake trout subjected to a single sea lamprey attack died most frequently in late summer, when temperatures exceeded 15.6°C. Christie and Kolenosky (1980) concluded that wounding and sea lamprey induced mortality of lake whitefish (*Coregonus clupeaformis*) in Lake Ontario peaked in August. Collectively, the evidence suggests that sea lamprey induced mortality does not occur at a constant rate, but is seasonal and strongly pulsed.

For lake trout longer than 430 mm, natural mortality from causes other than sea lamprey predation may be negligible in Lake Ontario. Most carcasses were recovered in the fall, when the increase in natural mortality was clearly caused by a pulse of sea lamprey induced mortality. Natural mortality from other causes was probably less seasonal and occurred at a low rate that resulted in recovery of only one unwounded fish in our bottom trawls. Unless substantial natural mortality unrelated to sea lamprey attacks occurred during winter, when our sampling effort was small, total annual mortality of lake trout in Lake Ontario probably had two major components: fishing mortality and sea lamprey induced mortality. Also, because there was no commercial fishing for lake trout in U.S. waters, sport-fishing harvest was the principal form of fishing mortality. Creel surveys indicated that the harvest by Lake Ontario anglers fishing in U.S. waters (with 95% confidence intervals in parentheses) was 96 000 (57 000 – 135 000) lake trout in 1984 and 84 000 (66 000 – 103 000) in 1985 (Cape Vincent Fisheries Station, unpubl. reports). Our estimates of the numbers of sea lamprey induced lake trout deaths varied widely during the falls of 1982–85 (Table 2) but in some years approached the level of angler harvest suggested by the 1984 and 1985 creel surveys. If, as we judge, the values in Table 2 were conservative estimates of the number of lake trout killed, predation by sea lampreys could have exceeded angler harvest in years of high sea lamprey abundance. The data presented here suggest that even though the sea lamprey has been controlled in Lake Ontario since 1971, it has continued to be a significant source of mortality of lake trout.

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Scaled Chrysophytes (Chrysophyceae) as Indicators of pH in Sudbury, Ontario, Lakes

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Surface sediment chrysophytes (Chrysophyceae) of 30 Sudbury, Ontario, lakes were analyzed to investigate the relationship of these algal microfossils with the limnological characteristics of the study lakes. The distribution of the majority of common chrysophyte taxa is correlated closely with lake water pH, pH-related factors, or both. *Chrysodidymus synuroideus*, *Synura echinulata*, *Mallomonas hamata*, *M. acaroides* var. *muskokana*, and *Chrysosphaerella longispina* appear to be indicators of strongly to moderately acidic waters whereas *M. pseudocoronata* and *M. caudata* are indicators of circumneutral to alkaline waters. Using multiple regression of pH indicator chrysophyte assemblages against the measured lake pH, a calibration equation was developed to compute chrysophyte-inferred pH. Chrysophyte-inferred pH values were closely related with measured lake water pH ($r^2 = 0.74$). The study indicates that in the absence of historical pH data, stratigraphic analysis of scaled chrysophytes would provide useful information about the history of lake acidification in the Sudbury area. There is some indication that high metal concentrations, in addition to pH, may influence chrysophycean species distribution in acidic lakes.

Les auteurs ont analysé les chrysophytes (Chrysophyceae) présents dans les sédiments de surface de 30 lacs de la région de Sudbury (Ontario) afin d'étudier la relation entre ces algues microfossiles et les caractéristiques limnologiques des lacs. La répartition de la majorité des taxons des chrysophytes les plus courants présentait une corrélation étroite avec le pH des eaux des lacs, les facteurs liés au pH, ou avec les deux. Les *Chrysodidymus synuroideus*, *Synura echinulata*, *Mallomonas hamata*, *M. acaroides* var. *muskokana* et *Chrysosphaerella longispina* semblent être indicateurs d'une eau fortement ou modérément acide, tandis que *M. pseudocoronata* et M. *caudata* indiquent la présence d'une eau peu acide ou alcaline. Les auteurs ont obtenu une équation d'étalonnage par régression multiple des assemblages de chrysophytes indicateurs du pH en fonction de la valeur mesurée du pH afin de pouvoir calculer les valeurs du pH reflétées par les chrysophytes. Ces valeurs se rapprochaient de celles mesurées ($r^2 = 0{,}74$). L'étude a montré qu'en l'absence de données historiques sur le pH, l'analyse stratigrahique des chrysophytes permettait d'obtenir des renseignements utiles sur l'évolution de l'acidification des lacs de la région de Sudbury. Selon certains indices, de fortes concentrations de métaux pourraient, en plus du pH, influer sur la répartition des espèces de chrysophycées dans les lacs acides.

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In the absence of past water chemistry data, sediment diatoms have been used widely to predict the rate and extent of lake acidification in North America and Europe (Battarbee 1984; Charles and Norton 1986; Dixit et al. 1987). The pH indicator potential of diatoms is well established (Charles 1985; Dixit 1986a; Dixit et al. 1988). However, the reliability of pH reconstruction can be questioned, as with any other paleolimnological interpretation, if it is based solely on one type of indicator. To some extent this deficiency can be corrected by exploiting the fact that the distribution of scaled chrysophytes is also correlated with lake water pH (Smol et al. 1984a, 1984b; Steinberg and Hartmann 1986).

Scaled chrysophytes (Chrysophyceae, Mallomonadaceae) constitute a group of flagellated algae common in lakes (Kristiansen 1975; Takahashi 1978). They are covered by species-specific, siliceous scales that are preserved over a wide range of lake conditions (Smol 1980). Because the methodology of preparation of scales from sediments is similar to diatoms and they can be enumerated on the same slide in conjunction with diatoms, scales can be incorporated easily in pH reconstruction studies where diatoms have been used.

The objective of this study was to investigate the pH-indicator potential of scaled chrysophytes for lakes in the Sudbury, Ontario, area. The approach that we adopted was to calibrate the distribution of surface sediment chrysophytes of 30 lakes against the limnological characteristics of lakes giving particular attention to lake water pH. Such a study is required prior to utilizing the stratigraphic analysis of chrysophytes to interpret the history of lake acidification in any specific region.

Study Area and Study Lakes

The study area extends to a distance of about 80 km from Sudbury (Fig. 1). The emissions from the metallurgical instal-

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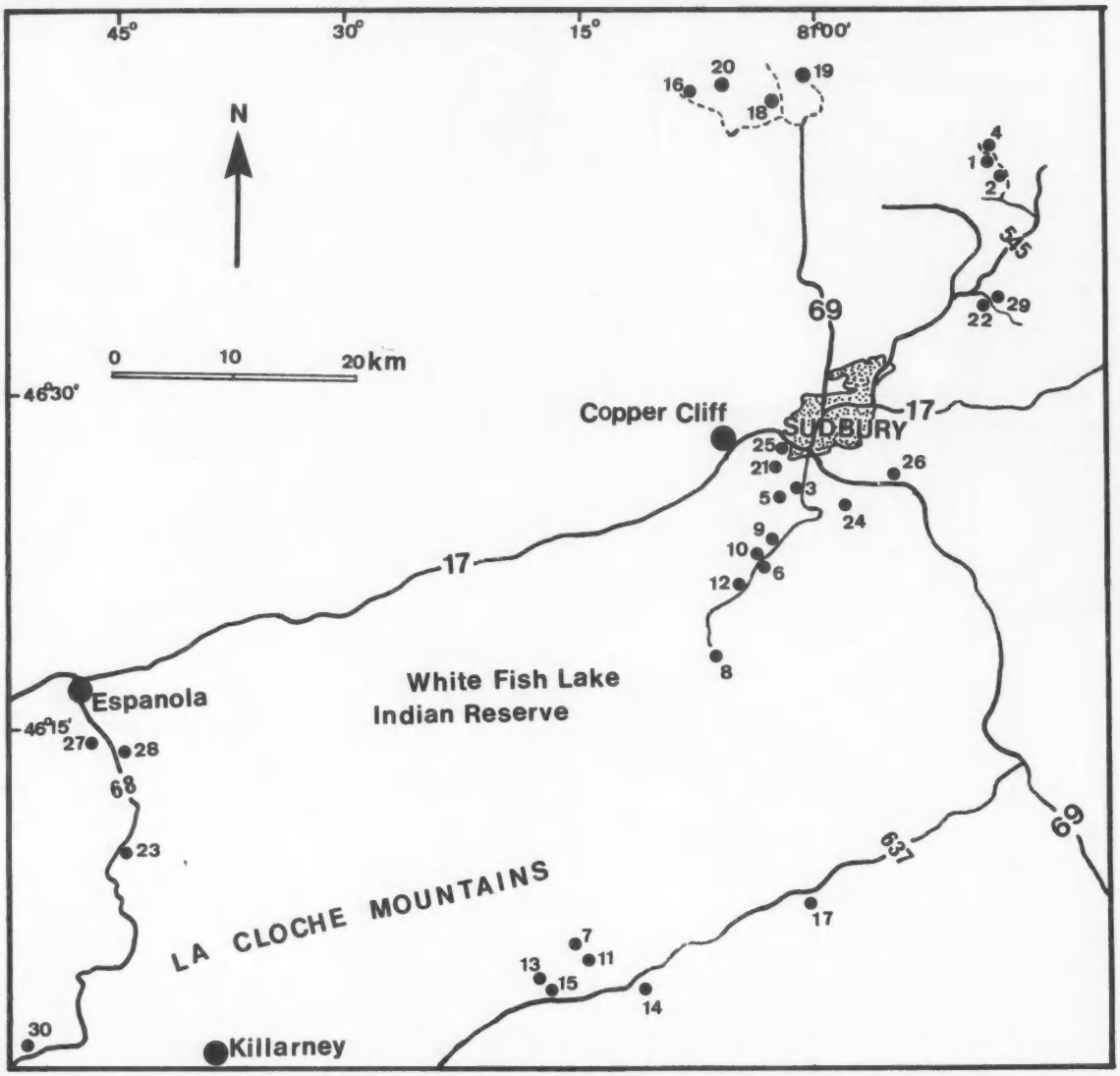

FIG. 1. Study area showing the location of 30 study lakes. Lake numbers correspond to lake names listed in Table 1.

lations in the Sudbury basin distinguish it as an area of major environmental interest. The area lies mainly on the Canadian Shield, underlain by Pre-Cambrian metamorphic, plutonic, and volcanic silicate rocks overlain by thin Pleistocene glacial deposits. The average precipitation pH in the study area is 4.3 (Chan et al. 1982) and the lake water pH is largely controlled by variation in geological setting and the prevailing wind pattern (Keller et al. 1980). On the basis of their wide range of pH, 30 lakes were selected for the study. Many of these lakes also receive large metal inputs from Sudbury smelters (Dixit 1986b).

Materials and Methods

In July 1984, a short sediment core (approximately 20–25 cm long) was collected from the deepest point of each of the 30 study lakes using a Kajak–Brinkhurst (K-B) gravity corer (Wildco Co. model 2402, Saginaw, MI, USA). The corer was modified to hold Plexiglas coring tubes of 6.3 cm internal diameter, a size that has been shown to be reliable in obtaining undisturbed cores of soft sediments (Evans and Lasenby 1984). On shore, the surface sediment (0–0.25 cm) of each core was carefully removed by extruding the core in the vertical position.

At the time when sediment cores were taken, an integrated water sample was collected from the deepest area of each lake. These samples were collected from the surface to the lower limit of the metalimnion in stratified lakes and from the surface to 1 m above the sediment surface in unstratified lakes. Lake water pH measurements were made in the field using a Fisher digital pH meter (model 119) with an accuracy of 0.01 pH unit. Within 12 h of sample collection, the total inflection point alkalinity was measured using the method outlined by the Ontario Min-

istry of the Environment (1979) and utilizing the Gran plot (Gran 1952). Water samples were analyzed for conductivity (at 25°C), total P, total N, soluble reactive Si, Ca, Mg, SO_4, Cl, Cu, Ni, Fe, Zn, and Al (Dixit 1986b). Because many study lakes were very acidic, their conductivity values were corrected for hydrogen ions (Sjörs 1950). Metal concentrations reported in this study are for total metals.

Chrysophyte scales were cleaned from the sediment samples using an acid digestion technique (Dixit 1986b) and cleaned subsamples were poured into Battarbee plates (Battarbee 1973). After all the water had evaporated, the microfossil-coated coverslips were mounted on glass slides using Hyrax mounting medium. The identification of scales was based on Takahashi (1978), Nicholls (1982), Wee (1982), and Smol et al. (1984b). Transmission electron microscopy was performed routinely to confirm the identifications of scales. An attempt was made to count and identify a minimum of 600 scales for each sample; however, in eight samples, only 98–210 scales were counted because the scales were not common in these samples. The counting was carried out in random fields under oil immersion at 1000× magnification.

All statistical analyses were made using SAS computer programs. To normalize the water chemistry data, conductivity, Ca, Mg, SO_4, Cl, Si, total P, Zn, Cu, Ni, Fe, and Al values were $\log_{10}$ transformed. Because many limnological variables were intercorrelated, they were factor analyzed. Subsequently, factors were related back to observations and factor scores were computed for lakes. The percent abundances of surface sediment chrysophytes were also $\log_{10}$ transformed. Log-transformed data are more ecologically meaningful because they give more importance to taxa that occur in moderate numbers and not to ones that are dominant or rare (Jensen 1980; Maarel 1982; Charles 1985). To help define the limnological factors affecting the distribution of common chrysophyte taxa, the correlations of the percent composition of chrysophyte taxa versus the factor scores from the factor analysis were used. An abundance weighted mean (AWM) pH was computed for common taxa in order to determine their optimum distribution along the pH scale using the following equation:

$$\text{AWM pH} = \frac{\sum_{n}^{i} (P_i \cdot X_i)}{\sum_{n}^{i} P_i}$$

where P_i = percent abundance of the taxon in sediment from lake i and X_i = measured pH of lake i.

Results and Discussion

The chemical and physical characteristics of study lakes are presented in Table 1. In addition to data gathered in 1984 for this study, 18 of these lakes were also sampled during the midsummers of 1981, 1982, and 1983 (Ontario Ministry of the Environment 1984). An evaluation of these data for 18 lakes (Dixit 1986b) suggested that there were no major year-to-year variations in the chemical characteristics of most study lakes over this period. For example, the variation in lake water pH was <0.25 pH unit for 66% of lakes and for the remaining lakes the difference was <0.5 pH unit. Moreover, only minor seasonal pH variations were observed in the lakes which were extensively monitored by the Ontario Ministry of the Environment (1982).

A correlation matrix of various chemical and physical variables (Table 2) indicated that low pH was significantly correlated with high Al, Fe, Ni, Cu, and Zn and low alkalinity, total N, and Ca. Similar to trace metals, SO_4 concentrations were also negatively correlated with pH. All trace metals considered here were positively correlated with each other. High trace metal concentrations in low-pH lakes have resulted from the leaching of metals from catchment soils and lake sediments during acidification and from direct atmospheric deposition of metals on lakes (Ontario Ministry of the Environment 1982).

Factor analysis was performed to identify groups of interrelated variables and to reduce the number of variables investigated (Table 3). The first factor, which explains 24.5% of the variance, has high positive loadings for metals (Zn, Cu, Ni, Fe, and Al) and SO_4 and negative loading for pH. This factor is mainly related to trace metals and pH. Low-pH lakes from this data set have high metal concentrations. The second factor explains 23% of the variance and has high positive loadings for conductivity, Ca, Mg, and Cl. This factor primarily reflects the ionic composition of the lakes. The third factor, explaining 18.8% of the variance, has high positive loadings for pH and alkalinity and a negative loading for Al. Although pH is also significantly related to factor 1, its relationship with factor 3 is much stronger. The dual relationship of pH is mainly due to the diverse set of lakes selected in this study. Although the first three factors have explained most of the variance (66%), the remaining variance is related to factors 4 (Si and Fe), 5 (total P), and 6 (Secchi depth).

A total of 24 taxa were identified from the surface sediments of 30 lakes (Table 4). The identifications of many of these taxa (20 species) were established using the transmission electron microscope (see Fig. 3 and 4). Among the taxa identified, only *Chrysodidymus synuroideus*, *Chrysosphaerella longispina*, *Mallomonas acaroides* var. *muskokana*, *M. caudata*, *M. crassisquama*, *M. hamata*, *M. pseudocoronata*, *Synura echinulata*, *S. petersenii*, and *S. sphagnicola* were common (i.e. >2% of the total scale counts in at least three lakes). The relationship between the distribution of common chrysophytes and lake water pH is presented in scatter diagrams (Fig. 2). Table 5 explains correlations between the relative abundances of common chrysophyte taxa and factor scores derived from the factor analysis of limnological variables.

Mallomonas crassisquama (Fig. 3a) was the most abundant taxon in the majority of study lakes. This is consistent with Nicholls' (1982) observations that *M. crassisquama* is the most common and widely distributed *Mallomonas* species in Ontario. The distribution of *M. crassisquama* in Sudbury lakes did not indicate any specific pH preference (Fig. 2), nor did it correlate with any other limnological variables (Table 5). This species can be considered as pH indifferent. Smol (1986) also found this taxon to be a pH generalist, but only in lakes with lake water pH > 5.0. The decline of *M. crassisquama* in core diagrams has been suggested as a definite marker of acidification (Steinberg and Hartmann 1986). The high percent abundance of *M. crassisquama* even in very acidic Sudbury lakes (e.g. Lake M2, pH = 3.08; 48% *M. crassisquama*) does not agree with observations that *M. crassisquama* declines in very acidic conditions (pH < 5.0). The difference in ecological requirement of this taxon emphasizes that such variation may occur between lake regions, and thus, autecological data from one region should not be applied to others without gathering such information for the specific study area.

Asmund and Kristiansen (1986) suggested that the body scales of thin-scaled populations of *M. crassisquama* cannot be distinguished from body scales of *M. acaroides*; however, this

TABLE 1. Some physicochemical characteristics of 30 study lakes in the Sudbury, Ontario, region.

Lake	pH	Alk. (μeq/L)	Cond. (μS/cm)	Ca (mg/L)	Mg (mg/l)	SO_4 (mg/L)	Cl (mg/L)	TN (μg/L)	TP (μg/L)	Si (mg/L)	(μ
1. M2	3.08	−832	1250	9.6	4.6	35.3	3.1	39	25.5	3.1	
2. M3	3.28	−525	546	13.5	3.8	29.3	2.7	72	21.0	2.2	
3. Silver	4.15	−71	330	7.8	4.1	39.1	38.6	128	3.5	1.7	
4. M1	4.38	−42	69	6.7	2.7	24.4	2.2	91	3.3	1.8	
5. Crooked	4.48	−33	81	4.8	1.9	18.2	2.6	106	7.0	1.6	
6. Clearwater	4.49	−32	76	7.8	0.9	20.6	5.5	36	5.0	1.5	
7. Ruth-Roy	4.58	−26	46	2.1	0.3	10.3	0.5	440	5.6	0.3	
8. Wavy	4.69	−20	47	1.9	1.0	12.1	2.9	138	5.9	1.2	
9. Lohi	4.74	−18	96	5.6	1.1	20.2	5.8	78	6.6	1.2	
10. Swan	4.85	−14	51	3.8	0.9	16.6	1.9	166	4.2	1.7	
11. Johnnie	5.11	−8	36	3.1	1.0	10.8	0.5	81	4.5	0.9	
12. Tilton	5.14	−7	53	5.6	1.3	14.5	3.6	54	7.8	0.7	
13. Terry	5.55	16	53	1.3	1.2	11.6	0.5	113	16.3	0.8	
14. Mahzenazing	5.55	10	39	1.9	1.2	10.2	1.4	225	13.0	1.1	
15. Carlyle	5.65	8	43	2.3	1.0	8.8	0.6	196	12.0	0.5	
16. Sans Chamber	5.79	18	53	4.6	0.9	14.3	0.5	336	16.1	0.7	
17. Attele	6.07	36	44	2.1	1.1	8.8	0.7	160	8.0	0.1	
18. Pigeon	6.17	104	38	4.5	4.1	12.8	0.5	87	11.5	0.4	
19. Joe	6.20	20	43	3.6	1.0	12.6	0.4	188	6.8	0.3	
20. Nelson	6.59	55	41	4.3	1.2	9.7	0.2	136	23.7	0.7	
21. Hannah	6.81	310	308	13.6	2.6	18.6	5.9	184	7.9	0.4	
22. Norway	7.25	303	81	12.6	3.6	12.9	0.7	326	18.1	0.3	
23. East Fox	7.26	243	332	19.6	4.4	5.4	6.6	278	21.6	1.1	
24. McFarlane	7.35	580	269	18.4	6.1	16.5	35.1	198	7.6	0.9	
25. Robinson	7.41	658	367	11.3	4.0	11.6	11.6	186	29.4	1.0	
26. Richard	7.62	334	174	15.1	4.9	13.6	15.6	254	13.6	1.4	
27. Griffin	7.71	693	154	21.4	2.6	8.1	10.6	376	33.5	0.7	
28. Anderson	7.81	452	97	13.7	6.6	6.1	6.0	265	9.0	0.5	
29. Boucher	8.12	985	434	17.7	8.1	14.2	13.4	369	12.8	1.7	
30. Lewis	8.29	2310	311	27.6	5.0	11.6	4.8	392	31.2	0.8	

Si (µg/L)	Zn (µg/L)	Cu (µg/L)	Ni (µg/L)	Fe (µg/L)	Al (µg/L)	Secchi (m)	Elev. (m)	Coring depth (m)
.1	210	409	2030	21 200	1 978	3	318	10
.2	311	324	964	14 000	15 000	3.5	318	2
.7	136	366	614	117	950	8	288	7
.8	99	52	409	580	93	6	318	5
.6	36	73	310	640	180	4	273	9
.5	41	159	195	47	310	10	283	21
.3	21	3	16	51	511	9.5	288	18
.2	12	18	83	124	140	7	261	10
.2	42	60	186	49	169	5.5	280	19
.7	12	240	86	386	27	6	288	8
.9	14	5	19	99	140	6	242	28
.7	25	35	137	54	117	6.5	259	14
.8	16	8	21	126	650	2	242	9
.1	12	4	23	132	91	3.5	194	18
.5	15	2	12	39	110	5	209	12
.7	15	4	15	146	71	8.5	349	15
.1	19	3	17	89	57	3	242	7
.4	28	5	25	132	49	5	393	5
.3	6	4	13	15	26	8	349	26
.7	4	1	5	31	40	7	349	24
.4	20	22	199	53	24	4.5	282	8
.3	123	527	85	154	29	3.5	288	9
.1	2	1	2	56	10	3	230	6
.9	11	10	62	47	38	3.5	226	18
.0	1	4	100	88	53	3	258	4
.4	1	3	147	34	23	3.5	258	10
.7	1	1	4	25	15	6	250	15
.5	1	1	5	95	25	3	242	18
.7	39	586	54	140	31	3	288	12
.8	1	1	1	76	7	5	179	11

did not cause any problem in this study, as we did not encounter thin-scaled populations of *M. crassisquama*. *Mallomonas acaroides* var. *muskokana* (Fig. 3b) was found frequently in lakes ranging in pH between 5.5 and 6.6 (Fig. 2), with the maximum abundance of 6% in Sans Chambre Lake (pH 5.8). The AWM pH for this taxon was 5.82. In addition to indicating a significant negative relationship with the pH factor, this species was negatively related with the conductivity factor (Table 5). The occurrence of *M. acaroides* var. *muskokana* in slightly acidic waters does not differ greatly from Smol's (1986) results, except that he found this taxon to be more abundant in acidic lakes. In our study, its low abundance in some acidic lakes may be partially due to their high conductivity (Table 1). Because in many acidic lakes, high conductivity may have been associated with excessive metal concentrations, metals may be responsible for the absence of *M. acaroides* in very acidic lakes (pH < 5.0).

In this study, the scales of *M. pseudocoronata* (Fig. 3f) were differentiated from *M. crassisquama*. The body scales of *M. pseudocoronata* are distinctively characterized by a broad unpatterned winglet which flares outside and forms the V-rib on each side of the distal portion of the scale and then tapers to a sharp pointed apex (Nicholls 1982). Posterior scales lack the flared wing, but possess thick, sharp, pointed spines. The separation of *M. pseudocoronata* from *M. crassisquama* was found to be necessary because these two species appear to have different pH indicator status. *Mallomonas crassisquama* is pH indifferent whereas the high percent abundance of *M. pseudocoronata* in lakes with pH about 7 and higher (Fig. 2) suggests that this taxon is an indicator of circumneutral to alkaline waters (AWM pH 7.35). Factor analysis also indicated a significant positive relationship with the pH factor (Table 5). Although Smol et al. (1984b) mentioned that the winglets of scales of *M. pseudocoronata* are often broken, thereby making them difficult to distinguish from *M. crassisquama*, in their reanalyzed data they successfully separated this taxon from *M. crassisquama* and found *M. pseudocoronata* to be alkaliphilous (J. P. Smol, Department of Biology, Queen's University, Kingston, Ont., pers. comm.).

The maximum percent abundance of *M. caudata* (Fig. 2) in some alkaline lakes (e.g. in East Fox (pH = 7.26) and Griffin (pH = 7.71) lakes, it comprised 24 and 14% of the total scale counts, respectively) and its AWM pH of 6.88 indicate that this taxon is an indicator of circumneutral to alkaline waters. Factor analysis provides similar results (Table 5). In earlier studies, *M. caudata* was recorded often from lakes ranging in pH from slightly acidic to alkaline (Cronberg and Kristiansen 1980; Wujek and Hamilton 1972; Smol 1986).

Similar to studies of Kristiansen (1981), Nicholls (1982), and Smol (1986), *M. hamata* (Fig. 3g) was found in greater abundance in acidic lakes (Fig. 2) with its AWM pH of 5.21. However, in Crooked, Clearwater, Wavy, Swan, Lohi, and Tilton lakes (pH range 4.5–5.2), *M. hamata* was not observed or only found sparsely. All these acidic lakes have higher Cu and Ni concentrations (Table 1) than the acidic lakes of the Killarney region (Ruth-Roy, Johnnie, Terry, Mahzenazing, and Carlyle) where *M. hamata* was found commonly. This may suggest that although *M. hamata* is an indicator of acidic waters, it may not succeed in acidic lakes with high metal concentrations. This hypothesis can be further substantiated by the factor analysis results (Table 5), indicating that the occurrence of *M. hamata* is closely associated with the pH as well as the metal–pH factor.

The scales of *C. longispina* (Fig. 3o and 3p) and *C. brevispina* were identified in study lakes. In general, *C. lon-*

TABLE 2. Correlation coefficient matrix for some physicochemical characteristics of 30 study lakes (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

	Elev.	Depth	Secchi	Al	Fe	Ni	Cu	Zn	Si	TP	TN	Cl	SO_4	Mg	Ca	Cond.	Alk.
pH	−0.31	0.16	−0.29	−0.81***	−0.56**	−0.53**	−0.51**	−0.73***	−0.53**	0.46*	0.69***	0.23	−0.57**	0.45*	0.49**	0.10	0.81***
Alk.	−0.45*	0.23	−0.09	−0.72***	−0.49**	−0.47**	−0.37*	−0.63***	−0.36*	0.20	0.65***	0.29	−0.34	0.36*	0.46*	0.06	
Cond.	−0.07	0.05	−0.39*	0.31	0.47**	0.46*	0.34	0.09	0.54**	0.38*	0.05	0.72***	0.45*	0.76***	0.78***		
Ca	−0.13	0.27	−0.23	−0.22	0.14	0.14	0.08	−0.22	0.23	0.36*	0.33	0.73***	0.18	0.85***			
Mg	−0.07	−0.19	−0.50**	−0.09	0.29	0.31	0.09	−0.10	0.29	0.39	0.14	0.63***	0.19				
SO_4	0.30	−0.21	0.22	0.66***	0.54**	0.82***	0.76***	0.68***	0.62***	−0.29	−0.49*	0.36					
Cl	−0.31	−0.31	−0.12	−0.03	0.04	0.38*	0.18	−0.14	0.35	−0.04	0.13						
TN	−0.30	0.19	−0.18	−0.55**	−0.38*	−0.47**	−0.34	−0.50**	−0.36*	0.33							
TP	−0.07	0.64***	−0.53**	−0.08	0.15	−0.30	−0.29	−0.35	−0.04								
Si	0.11	−0.06	−0.11	0.58***	0.76***	0.64***	0.59***	0.42*									
Zn	−0.41*	−0.21	0.08	0.71***	0.60**	0.71***	0.81***										
Cu	0.34	−0.36*	0.07	0.53**	0.55**	0.85***											
Ni	0.33	−0.18	0.02	0.63***	−0.58***												
Fe	0.22	0.06	−0.36*	0.74***													
Al	0.23	0.16	−0.04														
Secchi	0.32	0.36*															
Depth	0.16																

TABLE 3. Varimax rotated factor loadings for some physicochemical characteristics of 30 study lakes and the percent variance explained by each factor.

Variable	Factor 1	2	3	4	5	6
pH	−0.47	0.35	0.65	−0.40	0.19	−0.2
Alkalinity	−0.27	0.27	0.82	−0.16	0.09	0.09
Conductivity	0.24	0.86	−0.14	0.18	0.29	−0.08
Ca	−0.01	0.88	0.32	0.08	0.19	−0.02
Mg	0.06	0.82	0.25	0.14	0.18	−0.30
SO_4	0.77	0.32	−0.24	0.21	−0.06	0.36
Cl	0.07	0.91	0.01	0.00	0.26	0.04
TN	0.12	0.00	−0.05	−0.01	−0.09	0.19
TP	−0.28	0.23	0.04	0.02	0.86	−0.24
Si	0.37	0.33	−0.25	0.79	−0.07	0.02
Zn	0.86	−0.18	−0.30	0.13	−0.06	−0.08
Cu	0.91	0.12	−0.04	0.20	−0.11	−0.01
Ni	0.77	0.34	−0.32	0.16	−0.24	−0.26
Fe	0.47	0.13	−0.34	0.67	0.24	−0.26
Al	0.55	−0.03	−0.72	0.29	0.16	0.06
Secchi	0.01	−0.23	0.02	−0.08	0.28	0.82
Depth	0.16	0.06	−0.16	−0.06	0.19	0.32
Elevation	0.26	−0.11	−0.15	0.02	0.04	0.15
% variance	24.52	23.03	18.78	9.56	7.76	7.2
% cumulative variance	24.52	47.55	66.33	75.89	83.65	90.85

TABLE 4. Chrysophyte taxa identified in the surface sediment samples of 30 study lakes. The abundance weighted mean pH and pH groupings of taxa are also presented.

	AWM pH	pH Group
Chrysodidymus synuroideus Prowse	4.57	1
Chrysosphaerella brevispina Korsh. *em.* Harris *et* Bradley		
C. longispina Laut. *em.* Nicholls	5.39	2
Mallomonas acaroides var. *muskokana* Nicholls	5.82	3
M. akrokomas Ruttner	7.00	4
M. allorgei (Defl.) Conrad	6.18	4
M. caudata Iwanoff *em.* Krieger	6.88	4
M. crassisquama (Asmund) Fott	5.82	3
M. cf. *cratis* Harris *et* Bradley	7.81	5
M. cf. *doignonii* Bourrelly	5.93	3
M. galeiformis Nicholls	5.66	3
M. hamata Asmund	5.21	2
M. hindonii Nicholls	5.26	2
M. lelymene Harris *et* Bradley	5.74	3
M. papillosa Harris *et* Bradley	7.26	5
M. punctifera Korsh.	7.76	5
M. pseudocoronata Prescott	7.35	5
M. transsylvanica Peterfi *et* Momeu	6.46	4
Synura curtispina (Peterson *et* Hansen) Asmund	7.14	5
S. echinulata Korsh.	4.54	1
S. petersenii Korsh.	6.33	4
S. sphagnicola Korsh.	5.31	2
S. spinosa Korsh.	6.26	4
S. uvella Stein *em.* Korsh.	6.30	4

gispina was more abundant. Scales of this taxon (AWM pH 5.39) were found to be more abundant in lakes ranging in pH between 4.3 and 5.8 (Fig. 2). Factor analysis also indicates that this species is significantly related with pH (Table 5). Cronberg and Kristiansen (1980) found *C. longispina* as a characteristic taxon of oligotrophic humic lakes (pH range 5.47–6.23), and Peterfi (1967) found it in slightly acidic waters. Based on these observations it is possible to categorize *C. longispina* as an indicator of slightly acidic waters. However, *C. brevispina* can be found under very different conditions, including eutrophic and alkaline waters (Asmund 1973; Cronberg and Kristiansen 1980).

The presence of *S. petersenii* (Fig. 4a) in 16 lakes with diverse pH values (Fig. 2) and the lack of any significant relationship with limnological factors (Table 5) suggest that this taxon can be classified as a pH-indifferent species. Earlier reports of *S. petersenii* are also from lakes of diverse chemical characteristics (Asmund 1968; Cronberg and Kristiansen 1980; Smol et al. 1984b).

Synura echinulata (Fig. 4b and 4c) was found to be most abundant in lakes with a pH < 5 (Fig. 2). Its AWM pH was 4.54. The occurrence of *S. echinulata* was also related with the metal–pH factor demonstrating an additional significant negative relationship with the pH factor (Table 5). Based on our observations it is possible to suggest that *S. echinulata* is an indicator of acidic waters and, unlike *M. hamata*, the occurrence of this taxon is not adversely affected in acidic, metal-contaminated lakes. Smol (1986) found maximum abundance of *S. echinulata* at about pH 5, and Cronberg and Kristiansen (1980) observed it commonly in acidic lakes. The acidic pH indicator status of this taxon can be further supported by observing its increase in the recent sediments of some recently acidified Sudbury lakes (Dixit 1986b).

In this study, only in Terry, M1, and Mahzenazing lakes did the abundance of *S. sphagnicola* (Fig. 4g) exceed 2% of the scale sum (Fig. 2). Although all these lakes are acidic, the maximum abundance of *S. sphagnicola* (AWM pH 5.31) was in Terry Lake (22% of the total scale counts), the only humic-stained lake in this study. This might indicate that this taxon is more common in acidic humic-stained waters; however, such a relationship could not be depicted by factor analysis because of

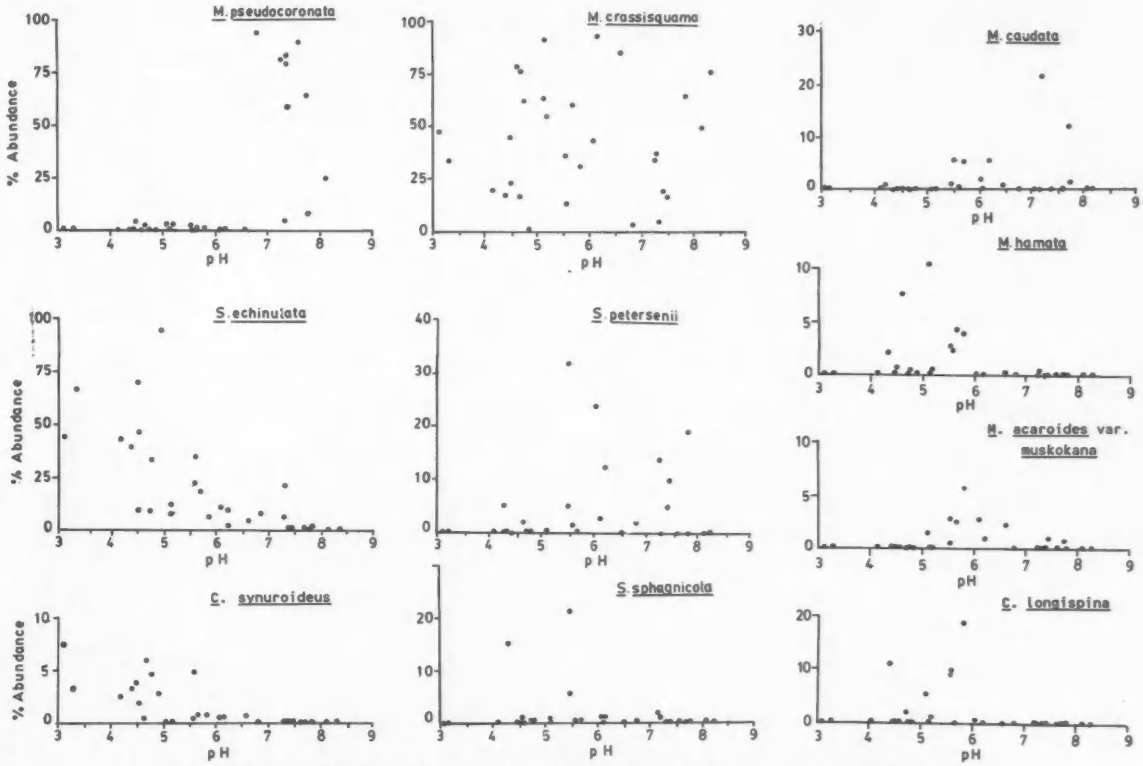

FIG. 2. Percent abundance of common chrysophyte taxa versus the measured pH of study lakes.

TABLE 5. Correlations of the percent composition of common chrysophyte taxa with the factor scores derived from the factor analysis of limnological characteristics (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

	Factor		
	1 Metal–pH	2 Conductance	3 pH
Chrysodidymus synuroideus	0.42*	—	−0.47**
Chrysosphaerella longispina	—	—	−0.39*
Mallomonas acaroides var. *muskokana*	—	−0.36*	−0.35*
M. caudata	—	−0.35*	0.36*
M. crassisquama	—	—	—
M. hamata	−0.35*	—	−0.41*
M. pseudocoronata	—	0.37*	0.48**
Synura echinulata	0.49**	—	−0.56***
S. petersenii	—	—	—
S. sphagnicola	—	—	—

the presence of only one acidic, humic-stained lake in this study. Cronberg and Kristiansen (1980) and Dürrschmidt (1980) observed that *S. sphagnicola* succeeds in acidic, humic-stained waters, and Harris and Bradley (1958) found that this taxon was closely associated with *Sphagnum* in shallow pools. Based on the limited autecological data available, *S. sphagnicola* appears to be an indicator of acidic, humic-stained waters. It is possible that in pH reconstruction studies the shift in relative abundance of this taxon may help to elucidate the shift in lake acidity from organic acid to strong mineral acid.

Although *C. synuroideus* (Fig. 4d) has been reported from North America (Wujek and Wee 1983), so far no autecological information exists on the taxon. In our study, *C. synuroideus* was usually common in lakes with pH < 5 (Fig. 2; AWM pH 4.57). In addition to a significant negative relationship with the pH factor, the positive relationship of this taxon with the metal–pH factor suggests that, similar to *S. echinulata*, *C. synuroideus* is not impacted negatively in acidic lakes containing high metal concentrations. The acidic occurrence of this taxon can be further substantiated from the surface sediment study of New England lakes where *C. synuroideus* was also found as a common chrysophyte in acidic lakes (S. S. Dixit and J. P. Smol, Department of Biology, Queen's University, Kingston, Ont., unpubl. data).

In order to fully utilize the pH indicator potential of chrysophytes, the next step is to quantify their relationship with lake water pH. Although several techniques have been developed to derive predictive pH equations using diatoms (Charles 1985; Davis and Anderson 1985; Dixit 1986a; Dixit et al. 1988), no such information exists for chrysophytes. In this study we have developed a calibration equation for chrysohytes using the multiple linear regression of pH indicator chrysophyte assemblages against the lake water pH. This technique has already been proven to be the best available method to compute diatom-inferred pH (Charles and Norton 1986; Dixit et al. 1988).

Chrysophyte taxa were grouped into 5 pH indicator assemblages based on their abundance weighted mean pH values

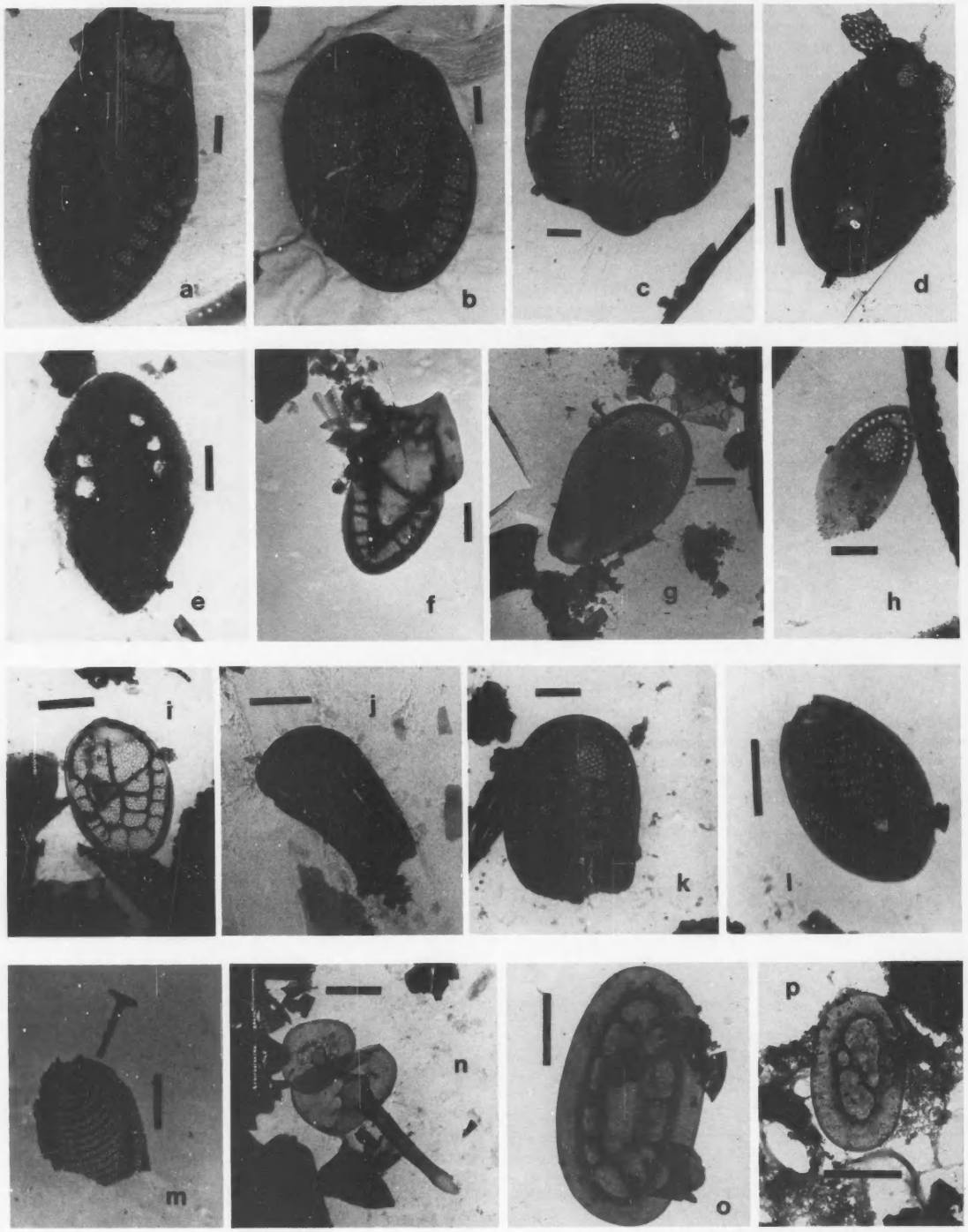

FIG. 3. Transmission electron micrographs of siliceous scales of (a) *Mallomonas crassisquama*, (b) *M. acaroides* var. *muskokana*, (c) *M. transsylvanica*, (d) *M. papillosa*, (e) *M. allorgei*, (f) *M. pseudocoronata*, (g) *M. hamata*, (h) *M. akrokomas*, (i) *M. hindonii*, (j) *M.* cf. doignonii, (k) *M. punctifera*, (l) *M. galeiformis*, (m) *M.* cf. *cratis*, (n) a spine of *Chrysosphaerella brevispina*, and (o and p) *C. longispina*. Scale bars represent 1 μm.

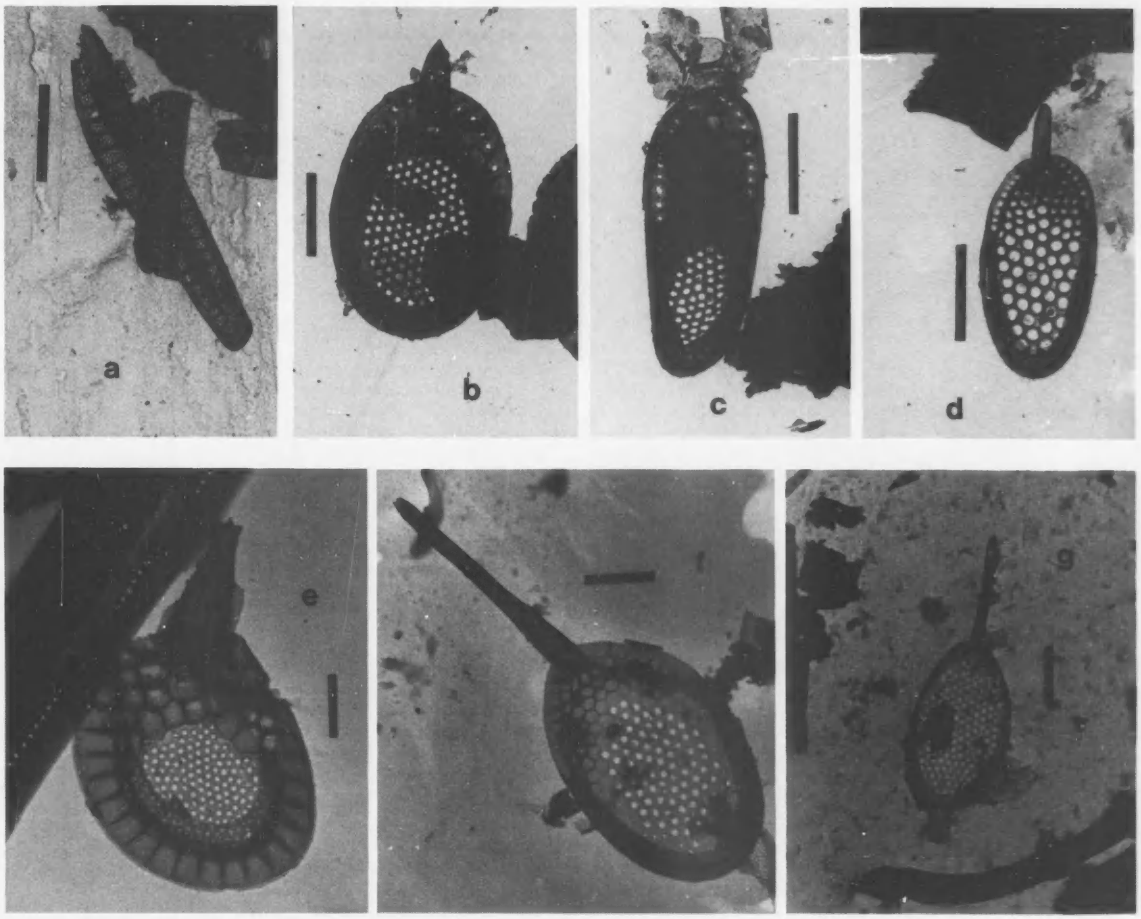

FIG. 4. Transmission electron micrographs of siliceous scales of (a) *Synura petersenii*, (b and c) *S. echinulata*, (d) *Chrysodidymus synuroideus*, (e) *Synura uvella*, (f) *S. spinosa*, and (g) *S. sphagnicola*. Scale bars represent 1 μm.

(Table 4): Group I, taxa with AWM pH < 5.0; Group II, taxa with AWM pH between 5.0 and 5.6; Group III, taxa with AWM pH between 5.6 and 6.0; Group IV, taxa with AWM pH between 6.0 and 7.0; Group V, taxa having AWM pH > 7.0.

Multiple linear regression analysis of these assemblages with measured lake water pH provided the following predictive pH equation:

$$\text{Chrysophyte-inferred pH} = 15.4416 - (0.1218 \times \%\ \text{Group I}) - (0.1064 \times \%\ \text{Group II}) - (0.0938 \times \%\ \text{Group III}) - (0.0854 \times \%\ \text{Group IV}) - (0.0752 \times \%\ \text{Group V}).$$

Using this calibration equation, chrysophyte-inferred pH was computed for each of the study lakes. A simple linear regression analysis of chrysophyte-inferred pH against the measured pH of lakes (Fig. 5) indicated a strong relationship ($r^2 = 0.74$). The standard error of this relationship suggests that an accuracy of ±0.36 pH unit can be achieved in predicting lake water pH using this technique.

The close association between lake water pH and percent abundance of many common chrysophyte taxa and significant relationship between measured and inferred pH suggest that these algal microfossils are sensitive indicators of lake water pH. Their stratigraphic analysis in Sudbury lakes would provide useful information about the pH-related changes in lakes resulting from the increase in metal mining and smelting activity in the Sudbury basin. In addition to providing complementary information where diatoms have been used to predict the past lake water pH, it is possible that in acidic lakes, chrysophytes are more sensitive indicators of pH because they occupy the pelagic habitat where chemical changes are first to occur. In contrast, diatoms primarily inhabit benthic habitats in acidic lakes (Almer et al. 1974; Charles 1985), where chemical changes related to acid inputs may be buffered by the sediments via cation exchange, humic substances, and dissolution of carbonates (Hongve 1978; Steinberg and Hartmann 1986).

In addition to highly acidic precipitation, lakes in the Sudbury area also receive high metal inputs (Keller et al. 1980). It is possible that in acidic lakes, metal concentrations may be important to chrysophytes. To date, knowledge of chrysophyte–metal interaction is nonexistent, although metals can be

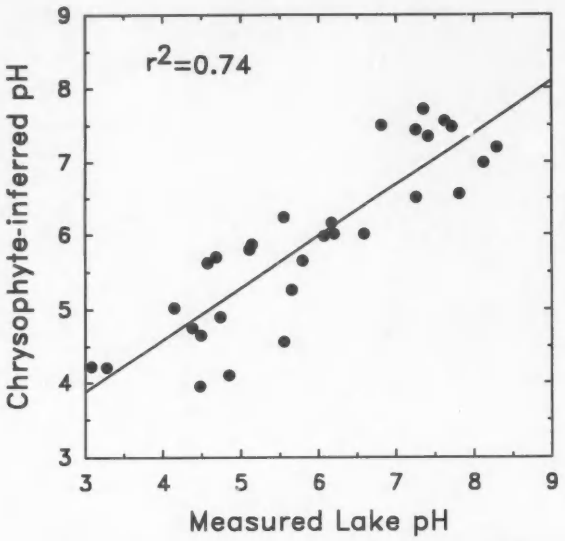

FIG. 5. Chrysophyte-inferred pH versus measured pH of study lakes.

toxic to other algae at high concentrations. Metal toxicity is influenced by pH, metal speciation, organic–metal complexation, metal precipitation, and alteration by other metals (Peterson et al. 1984; Campbell and Stokes 1985).

Conclusions

Surface sediment chrysophytes of 30 Sudbury lakes indicate that the distribution of many taxa is closely correlated with lake water pH, pH-related factors, or both. *Chrysodidymus synuroideus* and *S. echinulata* can be categorized as indicators of very acidic waters (pH < 5) whereas *C. longispina* and *M. acaroides* var. *muskokana* appear to indicate lake water pH ranging from 5.0 to 6.0. *Mallomonas pseudocoronata* and *M. caudata* indicate circumneutral to alkaline waters, and *M. crassisquama* and *S. petersenii* do not have any specific pH range. *Synura sphagnicola* may be an indicator of organic as opposed to mineral acidity. The close agreement between measured and chrysophyte-inferred pH provides additional evidence of the strong influence of pH on chrysophyte species composition. The calibration equation developed in this study will have wider applicability in reconstructing the pH history of lakes. These pH reconstructions will considerably aid in the understanding of pH-related changes in poorly buffered Sudbury lakes. Moreover the use of chrysophytes in association with already developed diatom-inferred pH methods for Sudbury lakes (Dixit et al. 1988) will enhance the accuracy of pH reconstructions. There appears to be some indication that in acidic lakes with elevated metal concentrations, the occurrence of chrysophyte taxa (e.g. *M. hamata*) may also be influenced by metal concentrations. Further research is underway to elucidate the combined effects of pH and trace metals on chrysophycean communities. Such an understanding will be helpful in fully utilizing the paleo-indicator potential of these planktonic algal microfossils.

Acknowledgments

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Ontogenetic Development of Growth Rates in Larval Pacific Herring, *Clupea harengus pallasi*, Measured with RNA–DNA Ratios in the Strait of Georgia, British Columbia

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During the spring of 1986, a cohort of Pacific herring, *Clupea harengus pallasi*, larvae was sampled for 36 d in the Strait of Georgia to measure growth rates using RNA–DNA ratios for individual larvae. Concurrent with the field study, a population of herring larvae was starved from hatching in captivity for comparison with those caught in the field. The mean RNA–DNA ratio at hatching for the starved larvae was close to 2 but it quickly rose to 3.4 by age 4 d. The mean RNA–DNA ratio subsequently dropped back to 2 and below by age 8 d, presumably reflecting the exhaustion of the endogenous food supply of the yolk sac. The mean RNA–DNA ratio at the calculated point-of-no-return was 2.06 which was very similar to the zero protein growth rate or what we define as the "critical ratio." Herring larvae from the field generally showed an increase in the RNA–DNA ratio over the 36 d from approximately 2 to 7 although the first 18 d showed more variation than the latter. There was no evidence of mass starvation ("critical period") for the 1986 year class but there was a noticeable drop in the growth rate during the change to exogenous feeding. We suggest that starvation probably only directly affected the developing larvae during a window of about 11 d. Frequency distributions of the RNA–DNA ratios are shown for larvae over time.

Au cours du printemps de 1986, les auteurs ont échantillonné pendant 36 d des larves de harengs du Pacifique (*Clupea harengus pallasi*) d'une même cohorte se trouvant dans le détroit de Géorgie afin d'en déterminer les taux de croissance. Ils ont utilisé, pour ce faire, le rapport entre l'ARN et l'ADN de chaque larve. Au cours de la même période, une population de larves de harengs a été conservée en captivité, sans être nourrie, afin de permettre la comparaison avec les valeurs obtenues sur le terrain. Le rapport moyen ARN–ADN à l'éclosion des larves témoins approchait 2, mais il a rapidement atteint 3,4 à l'âge 4 d. À l'âge 8 d, le rapport avait diminué à la valeur de 2 ou moins, ce qui reflétait probablement la fin de l'utilisation des sources alimentaires endogènes du sac vitellin. Le rapport ARN–ADN moyen au point calculé de non-rétablissement était de 2,06. Cette valeur est très semblable au taux de croissance zéro par manque de protéines ou de celui défini par les auteurs comme étant le « rapport critique ». Les larves étudiées sur le terrain ont présenté, de façon générale pendant les 36 d de l'étude, une augmentation du rapport ARN–ADN. Ce rapport passait de 2 à 7 et la variation était plus apparente au cours des 18 premiers jours. Rien n'indiquait que la classe de 1986 avait souffert d'un manque de nourriture grave (période critique) mais on a noté une chute appréciable du taux de croissance lors du passage à l'alimentation exogène. Les auteurs émettent l'hypothèse que le manque de nourriture n'a directement affecté les larves en croissance qu'au cours d'une période limitée à 11 d environ. Les distributions de fréquences des rapports ARN–ADN des larves en fonction du temps sont présentées.

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In 1914, Hjort hypothesized that recruitment to a year class of fish was determined in the very earliest larval stages and a critical period occurred during the switch from endogenous feeding on the yolk sac to exogenous feeding on plankton where starvation-induced mortality was high. Despite a profusion of studies designed to test this hypothesis, no firm conclusion has been reached (see May 1974 for review). One of the major problems has been sampling a rapidly dispersing larval fish population with enough resolution to detect a sharp change in the mortality rate over the short period of time in the feeding transition phase. Because of the resolution problem, research has been conducted to establish whether larvae are indeed starving in the field. This has been done for herring (Hempel and Blaxter 1963; Chenoweth 1970; Ehrlich et al. 1976; McGurk 1985), cod (Koslow et al. 1985; Neilson et al. 1986), plaice (Shelbourne 1957; Ehrlich et al. 1976), northern anchovy (O'Connell 1976, 1980), and jack mackerel (Theilacker 1978, 1986).

Three basic methods have been used to diagnose nutritional condition in larval fish: morphometric, histological, and chemical. While each has certain attributes and advantages, the chemical condition factors are probably the most sensitive, as

changes in the fish would occur first at the cellular level, then the tissue level, and finally the organism level. Some studies report significant differences in certain chemical constituents in as little as 2–4 d (Ehrlich 1974; Buckley 1981). A chemical method used to measure the state of growth and nutritional condition in larval fish is the ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA). Use of this ratio is based on the premise that while the DNA per cell is approximately constant, the amount of RNA in the cell is directly proportional to the amount of protein synthesis occurring. Several studies have shown that there is a linear relationship between the rate of protein synthesis and the RNA–DNA ratio (Haines 1973; Buckley 1979, 1980, 1982, 1984). Buckley (1984) found that if the RNA–DNA ratio was combined with data on water temperatures, 92% of the variance in the protein growth rate could be explained. This technique is particularly appropriate for studying the short-term growth of larval herring as well as other larvae. Ehrlich (1974) has shown that herring larvae produce predominantly protein and some carbohydrate instead of triglycerides during their early growth stages (up to 20 mm). The resolution of this method then allows one to follow changes in growth rates on a fine time scale.

The objective of this study was to follow the growth rates of a cohort of Pacific herring, *Clupea harengus pallasi*, larvae over time in the field using RNA–DNA ratios on individual fish. The hypothesis being tested was that herring larvae experience a "critical period" (sensu Hjort 1914) at the onset of exogenous feeding which will be reflected as a sharp drop in the mean growth rate. In addition, individual larvae will be analyzed to determine the RNA–DNA ratios by morphological stage and the frequency distribution of RNA–DNA ratios over time.

Materials and Methods

Field Study

This study was conducted from March 31 to May 6, 1986 (Calendar Days 90–126) in Lambert Channel (49°30′N, 124°43′W) in the Strait of Georgia, British Columbia (Fig. 1). The area is at present one of the major spawning areas for Pacific herring in the Strait of Georgia (Hourston 1980). Spawning usually occurs on the eastern side of Denman Island, with occasional spawnings occurring on the northern and eastern side of Hornby Island. Three sampling sites were chosen to follow the emerging cohort of herring larvae (Fig. 1). This work is part of a larger study examining the dispersion and feeding dynamics of larval Pacific herring (S. M. C. Robinson, in prep.).

Temperature and salinity measurements were taken with a portable temperature/salinity probe (Autolab model 602) at each of the sampling sites. The mean temperature at a depth of 5 m increased from 8.4°C on Calendar Day 92 to 9.4°C on Calendar Day 126. This depth was chosen based on the observation that well over 50% of the larvae are found in the top 10 m and also the temperature gradient from 5 to 30 m was generally on the order of 0.5°C due to the mixing of the water column at this time of year (S. M. C. Robinson, unpubl. data). The salinity at 5 m ranged from 27.3 to 28.9 ppt, with a mean of 28.3 ppt (SD = 0.4, n = 15).

Larvae were captured from a small (7 m) boat using 22-cm-diameter bongo nets with 350-μm Nitex mesh. Both frame and nets were black to reduce net avoidance (LeBrasseur et al. 1967). Single cycle oblique tows (e.g. down and up) at a towing speed of 1.5 $m \cdot s^{-1}$ were done to 50 m or the bottom, whichever was shallower. Tows were done during the daylight hours. A subsample of captured larvae (n = 6–23) were individually placed in numbered 1.5-mL microcentrifuge vials containing 1 mL of 20-μm filtered seawater. These were then frozen and stored in liquid nitrogen for later analysis. The larvae were alive when frozen and the whole capture and preserving procedure took less than 10 min. Larval lengths were corrected for shrinkage in the liquid nitrogen before analysis of the data.

Starvation Controls

A control study was done to compare the larval herring RNA–DNA ratios observed in the field study with those from larvae known to be starved. Eyed eggs from natural spawn of Pacific herring were collected from Denman Island (Fig. 1) and held in aerated seawater at 8°C until hatching. Larvae were then transferred to aerated 70-L containers filled with 20-μm filtered seawater. Temperature was regulated with an external water bath to reflect temperature patterns found in the field. The salinity was constant at 29.0 ppt. At approximately 3-d intervals, subsamples (n = 6–15) of the larval population were taken and treated as above for later analysis of RNA–DNA ratios.

Laboratory Analysis

All previous studies measuring RNA–DNA ratios in larval fish (Buckley 1984; Wright and Martin 1985; Fukuda et al. 1986; Buckley and Lough 1987; Clemmesen 1987) have used the modified Schmidt–Thanhauser technique which requires a minimum of 800 μg dry weight·$sample^{-1}$. An 800-μg Pacific herring larvae would be approximately 28 d old (S. M. C. Robinson, unpubl. data); therefore, estimates of individual variation in the RNA–DNA ratio are lost until well into the initial exogenous feeding stages. A more sensitive technique, suitable for measuring small amounts of nucleic acids, uses the LePecq and Paoletti (1966) fluorometric procedure, modified by Karsten and Wollenberger (1972, 1977). This technique is based on the enhanced fluorescence of nucleic acids after introduction of the dye ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthidinium bromide) and is sensitive to small amounts of nucleic acids at concentrations down to 0.05 μg·mL^{-1} for DNA and 0.1 μg·mL^{-1} for RNA. For this study, an individual larva was thawed, measured for standard length under a dissecting microscope to the nearest 0.2 mm, and staged morphologically according to Doyle (1977). The larva was then placed in 3 mL of ice-cold phosphate-buffered saline and homogenized and sonicated with a Polytron tissue grinder at 24 000 rpm for two 10-s treatments. Two replicates of 0.5-mL aliquots were taken and processed for determination of DNA and two for the total nucleic acids. Fluorescence of the sample was measured with a FOCI Ratio Fluorometer-2 (Farrand Optical Company Limited) on sample mode.

Two modifications were made in the Karsten and Wollenberger technique. Incubation time was increased from 20 to 30 min and the RNase concentration was increased from 50 to 100 μg·mL^{-1} for added assurance that essentially all the RNA would be hydrolyzed by the enzyme. Standard curves were done for both DNA and RNA. Processing time of the samples by two persons was one fish every 5 min after the initial time lag of 30 min due to the incubation period. Samples were randomly chosen each day from the liquid nitrogen storage tanks so systematic errors would not affect a particular size group.

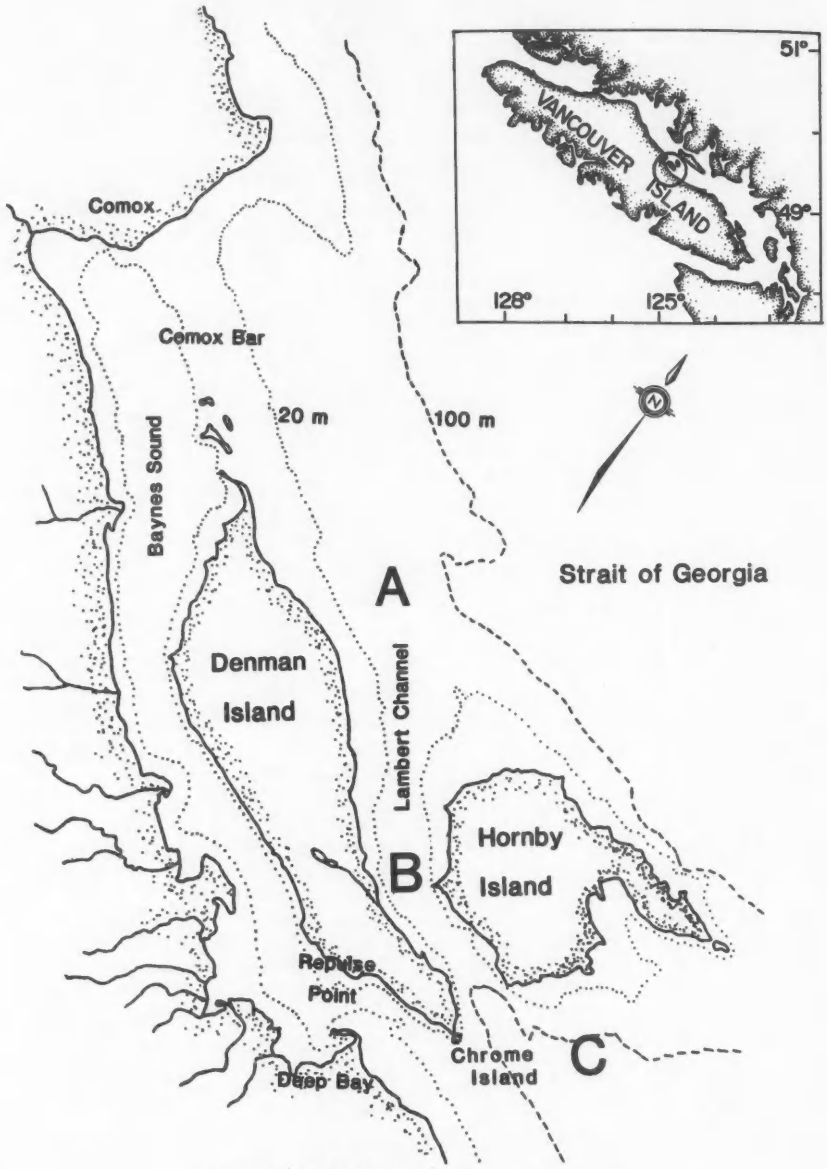

FIG. 1. Study site showing the three sampling areas.

Critical Ratio

Buckley (1984) examined the relationship between the instantaneous protein growth rate (the dependent variable), the RNA–DNA ratio (the independent variable), and water temperature. The relationship was linear ($r^2 = 0.92$) and was given as

(1) $G_{pi} = 0.93T + 4.75\text{RNA–DNA} - 18.18$

where G_{pi} is the protein growth rate (percent per day), T is water temperature (degrees Celsius), and RNA–DNA is the RNA–DNA ratio, Buckley's study involved temperature ranges from 2 to 20°C and eight species of marine fish larvae including Atlantic herring, *Clupea harengus harengus*.

Equation (1) can be further manipulated by setting G_{pi} equal to zero and then rearranging to give

(2) $\text{RNA–DNA} = R_{crit} = (18.18–0.93T)/4.75$

where R_{crit} is what we call the critical ratio and the other variables are the same as above. The critical ratio is the theoretical RNA–DNA ratio where there is no net protein growth in a larval fish at a specified temperature.

Results

Starvation Controls

There was a significant increase in the mean RNA–DNA ratio over the first 4 d from 1.82 to 3.37 ($p < 0.001$, Student's *t*-

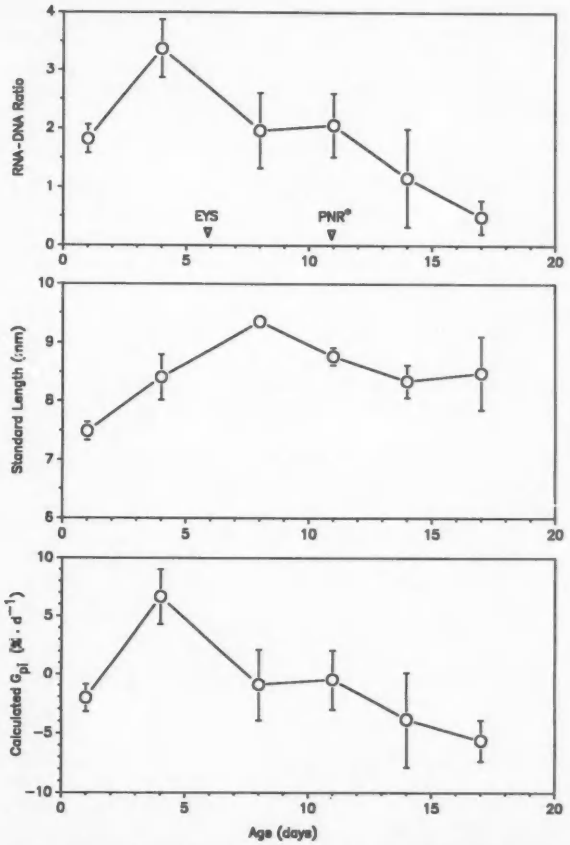

FIG. 2. Progression of the RNA–DNA ratio, standard length, and calculated protein growth rate of starved Pacific herring larvae over time in the starvation control tank. Bars represent 1 SE of the mean. EYS = end of yolk sac; PNR* = calculated point-of-no-return (McGurk 1984).

test) after which there was a decreasing trend in the mean ratio reaching 0.50 by the time the last larvae had died (Fig. 2). Marked on the figure is the observed end of yolk sac stage (EYS) and the point-of-no-return (PNR) calculated from McGurk (1984) for Pacific herring at these temperatures. At the calculated PNR of 11 d, the mean ratio was 2.06.

The mean standard length of the population increased for 8 d, after which it slowly decreased leveling out around day 14 at approximately 8.5 mm (Fig. 2). The reduction in length occurred as the RNA–DNA ratio reached the critical ratio of 2. Variation in the standard length between sampling periods was generally less than that observed in the RNA–DNA ratios.

The protein growth rates calculated from equation (1) show the same pattern as the RNA–DNA ratios due to the direct relationship between them. The mean values ranged from −5.6 to 6.7% protein increase·d^{-1}, while individual growth rates ranged from −10.0 to 17.3%·d^{-1}.

Field Study

Size frequency histograms of captured herring from Calendar Day 90 (March 31) to Calendar Day 119 (April 29) indicate that there is one major cohort of larvae resulting from the spawning

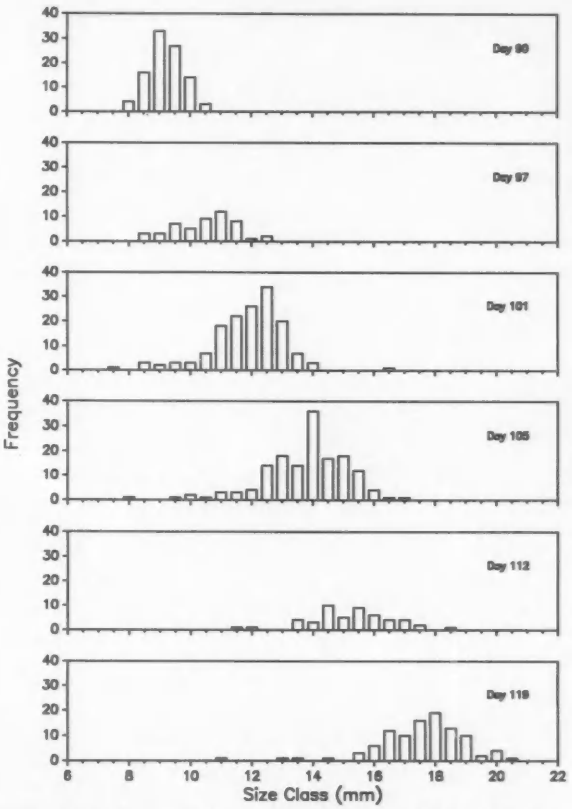

FIG. 3. Size–frequency histograms of larvae sampled from Lambert Channel from Calendar Day 90 to 119 in 1986.

adults (Fig. 3). Traces of larvae from a second spawning wave appear approximately 2 wk after the first, but the cohort was negligible in numbers. The linear growth rate of the larvae based on progression of modes was 0.31 mm·d^{-1}.

The mean RNA–DNA ratio for the wild population tended to increase over time from approximately 2 at Calendar Day 90 to 7 at Calendar Day 126 (Fig. 4). During the first half of the study period, from Day 90 to Day 108, the variance in the RNA–DNA ratio was significantly higher ($p < 0.001$, F-test) than it was in the latter half from Day 109 to Day 126. The progression of the mean RNA–DNA ratios over time for the three areas were different (e.g. on Calendar Day 106, Area A was significantly greater than Area B, $p < 0.05$), but all three areas gradually assumed similar values by Calendar Day 126. Except for Area C on Calendar Day 90, the mean RNA–DNA ratios of the field-caught larvae were consistently above the critical ratio.

The percentage of larvae below the calculated critical ratio in each of the three areas was found to be highest in the early periods (Table 1). In all the areas, there was a fairly rapid decrease in the percentage below the critical ratio, and by about Calendar Day 105, there were no larvae exhibiting a loss in growth.

The fluctuations, such as were found in the RNA–DNA ratios, were not as apparent in the mean standard lengths which increased over time in all areas, although not at continuous rates (Fig. 4). Overall, there was a positive correlation ($r = 0.67$, $p < 0.01$) between the standard length and the RNA–DNA ratio.

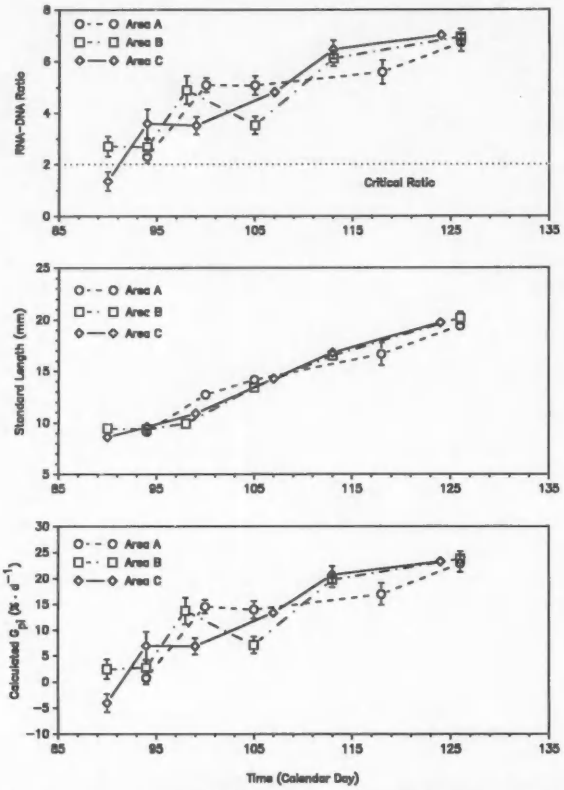

FIG. 4. Progression of the RNA–DNA ratio, standard length, and estimated protein growth rate (calculated from equation (1)) of field-caught Pacific herring larvae over time from the three sampling areas. The calculated critical ratio is plotted for reference. Bars represent 1 SE of the mean.

However, when this relationship was examined for each sampling period, except for one date (Calendar Day 117), there were no significant correlations between standard length and the RNA–DNA ratio ($p > 0.05$). While there may be no direct demonstrable relationship between ratio and length, a past history of high ratios results in larger larvae. Larvae from Area A appeared larger at first but as the ratios from Area B and Area C increased over Area A the mean sizes in Areas B and C also increased over those from Area A.

The mean RNA–DNA ratios for all areas combined were found to increase with each successive morphological stage except for stage 2a, the first feeding stage, where it dropped by about 23% compared with stage 1c (Table 2). The mean RNA–DNA ratio of stage 2a was significantly different from that of stage 2b ($p < 0.05$, Student's t-test) but not from stage 1c ($p > 0.05$, Student's t-test). The mean standard length also increased consistently with each morphological stage. In the early yolk sac phase (stages 1a and 1b), about 50% of the larvae were below the critical ratio, but this percentage dropped to zero by the end of the yolk sac stage 1c. The percentage sharply increased at stage 2a and then fell to much lower levels at successive stages.

Initially, the shape of the frequency distributions of the RNA–DNA ratios of the three areas was skewed right (Fig. 5). Because of logistic sampling problems, Calendar Day 90 was only composed of Areas B and C, and two periods (Calendar Day 105 + 107 and Calendar Day 124 + 126) had to be combined in order to show all three areas. By Calendar Day 105 + 107 the shape of the distribution was slightly skewed left but shifted to a strongly skewed left and truncated distribution by Calendar Day 124 + 126. The mode moved right over time and the range of values initially increased and then decreased.

Discussion

Starvation Controls

The calculated protein growth rates (percent per day) of the larvae quickly dropped to zero after exhaustion of their yolk sac, although the frequency at which the larvae were sampled was not high enough to demonstrate this unequivocally. The drop to zero of the growth rate was then followed by a reduction in length as the effects of inadequate nutrition presumably began to occur (Fig. 2). This pattern of shrinkage in standard length was similar to that found by Blaxter and Hempel (1963) for Atlantic herring larvae. The RNA–DNA ratio values and the initial increasing and then decreasing pattern of the ratio were very similar to those found by Clemmesen (1987) for Atlantic herring larvae; however, the drop in the ratios of her fish appeared to happen about day 4. The difference may be due to the higher rearing temperatures she used (14.9°C) or lower yolk reserves in the larvae. The duration of the yolk sac stage in this study was comparable with several other studies on captive larval Pacific herring (Schnack 1981; McGurk 1984).

Before day 8 the coefficient of variation (standard deviation × 100/mean) was approximately 50%; however, from day 8 onwards the coefficient of variation ranged from 79 to 191%, suggesting that the herring larvae are experiencing starvation effects. Blaxter and Hempel (1963) defined the point where a larva, deprived of food, is unable to feed even if suitable food becomes available as the PNR. For water temperatures used in this study, McGurk (1984) calculated that it took starved Pacific herring larvae 11 d to reach the PNR. This value is comparable with findings on Atlantic herring larvae (Blaxter and Hempel 1963; Blaxter and Ehrlich 1974). In our study, the mean RNA–DNA ratio at 11 d (from McGurk 1984) was 2.06, almost identical to the calculated critical ratio (2.03) derived from Buckley (1984). Clemmesen (1987) starved Atlantic herring

TABLE 1. Percentage of Pacific herring larvae having RNA–DNA ratios below the critical ratio R_{crit} during the sampling period in each of the three sampling areas. — = no observation for that day.

	Calendar Day										
Area	90	94	98	99	100	105	107	113	118	124	126
A	—	52.2	—	—	0.0	5.3	—	—	0.0	—	0.0
B	50.0	36.4	8.3	—	—	13.3	—	0.0	—	—	0.0
C	72.7	33.3	—	23.5	—	—	0.0	0.0	—	0.0	—

TABLE 2. Mean and standard deviations of RNA–DNA ratios and standard lengths for larval Pacific herring in all areas combined by morphological stage (from Doyle 1977). R_{crit} is the percentage of larvae below the critical ratio. Stage 1 may be described as yolk sac larvae, stage 2 as post yolk sac preflexion larvae, and stage 3 as postflexion larvae.

		RNA–DNA ratio		Standard Length (mm)		
Stage	n	$\bar{x}$	SD	$\bar{x}$	SD	R_{crit} (%)
1a	27	2.25	1.49	8.56	0.87	59.3
1b	25	3.21	2.10	9.43	0.72	52.0
1c	7	4.81	2.81	10.65	0.79	0.0
2a	32	3.71	1.74	11.54	1.21	28.1
2b	22	4.67	1.23	13.11	1.06	0.0
2c	26	5.42	1.30	14.97	1.26	0.0
3a	18	5.57	1.86	16.75	1.53	5.6
3b	20	6.30	1.29	17.33	1.68	0.0
3c	11	7.23	0.69	19.41	3.13	0.0

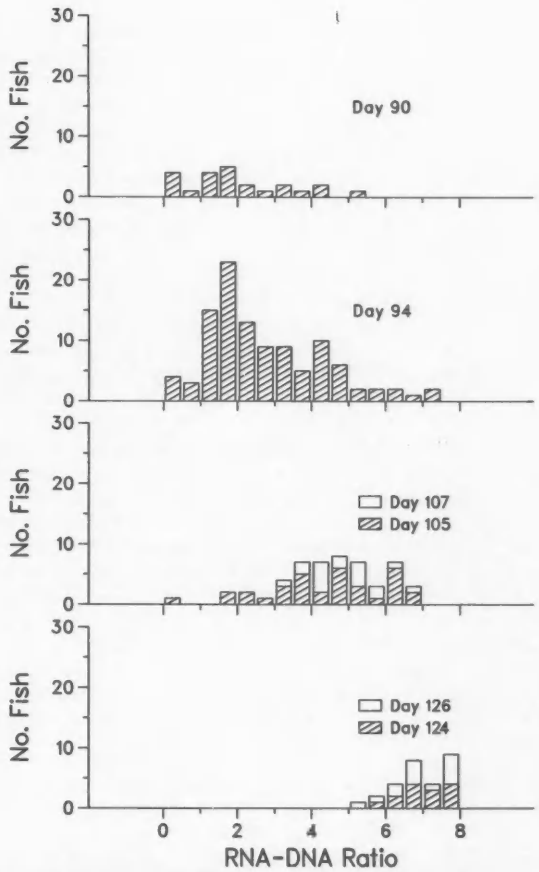

FIG. 5. Distribution of RNA–DNA ratios of Pacific herring larvae over time from the three sampling areas combined. Note that a few days had to be combined (see text).

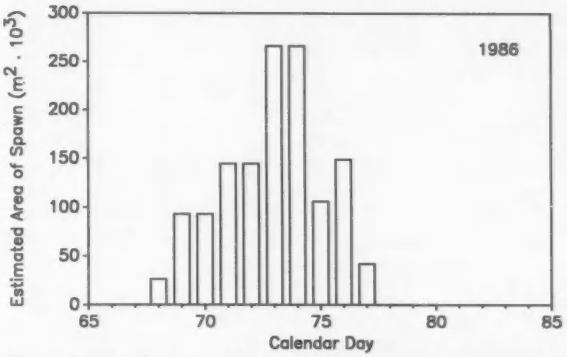

FIG. 6. Record of spawning dates and estimated amounts of spawn for Pacific herring in the Lambert Channel area during 1986. (data from D. Chalmers, Department of Fisheries and Oceans, pers. comm.).

larvae at different ages up to day 50 and found the RNA–DNA ratios seem to fall to approximately the same levels observed in this study. A similar pattern was found for larval striped bass, *Morone saxatilis*, although the experiment only lasted for 14 d (Wright and Martin 1985). Therefore, it appears that the critical ratio and the calculated PNR both have RNA–DNA ratios approximately equal to 2. More work should be done to clarify this possibility.

Field Study

Due to the daylight ichthyoplankton tows and the net size used, we recognize that some net avoidance of larger larvae undoubtedly occurred. If weaker or smaller larvae were selected for, the observed growth rates would be underestimates of the population and this study would represent a worst case scenario. However, the growth rates (millimetres per day) of the captured larvae compared favorably with other studies on Pacific herring larvae (Stevenson 1962; Schnack 1981; McGurk 1987), and therefore, we feel that most of the samples were representative of the population.

The one dominant mode and the almost imperceptible second mode in the length–frequency plots (Fig. 3) indicated that this study is representative of the growth and condition of the year class produced from this area. We have ignored the "daily" cohorts produced by the first spawning wave and assumed that they are all one cohort. The existence of a single dominant cohort is also supported by observations (D. Chalmers, Department of Fisheries and Oceans, pers. comm.) on the spawning dates and estimated amounts in this region (Fig. 6). Spawning was observed to occur over a period of 10 d at various locations with a peak at Calendar Day 73. No second wave was observed.

Although the trend was not consistent, the mean RNA–DNA ratios of larval herring in our study generally tended to increase (Fig. 4). This implies, during the first 36 d of life, that herring larvae in the field are not growing at a simple exponential rate because the RNA–DNA ratio, which is directly related to the instantaneous protein growth rate, increases with time. The fact that there was essentially no correlation of the RNA–DNA ratio with size at each sampling period suggests that early growth rates may be a function of larval age and/or the progression of the spring plankton bloom. Tanasichuk and Ware (1987) have suggested that higher growth rates of newly hatched larvae later in the season are why second spawning waves of herring do not produce larger larvae as their theoretical spawning model predicts.

The main hatch of larvae was estimated to start about Calendar Day 88 (inclement weather prohibited sampling from Calendar Days 87 to 89) and probably continued for 7 or 8 d. If we assume that the hatch began on Calendar Day 88 and the duration of the yolk sac stage was 6 d (McGurk 1984; this study), then the compulsory switch to exogenous feeding would start around Calendar Day 94. All three areas showed a levelling off or a decrease in the RNA–DNA ratio during the period Calendar Days 95–102 after a previous period of rapid increase (Fig. 4), suggesting that the larvae are undergoing a period of increased nutritional difficulties. This view of the larvae experiencing problems in the switch to exogenous feeding is also supported by the mean ratios for the morphological stages where there is a consistent increase in the ratios at each successive stage except for stage 2a (the onset of exogenous feeding; Table 2). The difference between the mean RNA–DNA ratios of stages 1c and 2a is not statistically significant, but we feel that this is due to the low sample numbers for stage 1c. In addition, 28% of the larvae were below the critical ratio in the first feeding stage (2a) compared with 0% at the end of the yolk sac stage (1c) (Table 2).

As the mean RNA–DNA ratio in each area continues to increase, it appears that by about Calendar Day 105 the larvae are at a stage where their growth increases well above the critical ratio and shows no signs of the earlier fluctuations. There were also no larvae found below the critical ratio after this date (Table 1). This suggests that if starvation is a significant factor in regulating population numbers in this cohort of larval herring, it will have to act primarily in a window of about 11 d. Herring would be affected from the end of the yolk sac stage on about Calendar Day 94 (assuming a yolk sac stage of 6 d) to about Calendar day 105. If the fish were unable to capture food, then the window of the PNR would be 6 d from Calendar days 99 to 105. A similar trend for larval Pacific herring, showing a brief initial period where larvae were diagnosed as starving, was found by McGurk (1985) using a multivariate condition factor. This view of a larval escape from food restrictions, with respect to starvation, after a brief window is supported by a study on jack mackerel, *Trachurus symmetricus* (Hewitt et al. 1985). They found that starvation was an important source of mortality rate only during a window of about 7 d during the first feeding stage. Ware and Lambert (1985) also found that mortality in Atlantic mackerel, *Scomber scombrus*, rapidly increased during the first feeding stages and then decreased a few days later.

This study supports some of the traditional views of the early life history feeding dynamics of herring. We found evidence that larvae do undergo some brief problems switching to a planktivorous diet and that this occurs during a small time window following exhaustion of the yolk sac. This is consistent with Hjort's (1914) original hypothesis. However, for this cohort, we can find no evidence to indicate that a "critical period" is present where a large increase in mortality occurs due to starvation during the switch to exogenous feeding. The mean RNA–DNA ratios during the estimated starvation window (Calendar Days 94–105) are significantly greater ($p < 0.05$, Student's t-test) than the critical ratio and the percentage of larvae below the critical ratio (Table 1) during this period is small and declines quickly.

When the mean RNA–DNA ratios from the field are compared with the critical ratio, one can see that there is a relatively rapid increase from values near the critical ratio on Calendar Day 90 to approximately 5 by day 100. It appears, then, that early yolk sac larvae (around day 90) grow very slowly or lose weight, as the mean RNA–DNA ratios are so close to the critical ratio. This characteristic may also be due perhaps to the production of biochemical compounds other than protein. It is possible that the increase in the ratio is overestimated, as slower growing larvae may experience higher mortality rates. The RNA–DNA ratios that we found in the field are higher than those reported by Fukuda et al. (1986) for Pacific herring larvae, Clemmesen (1987) for Atlantic herring larvae, and Wright and Martin (1985) for larval striped bass fed in the laboratory, but the differences may be due to population differences or the fact that fish larvae are notoriously difficult to raise normally in captivity. Although there are very few studies on RNA–DNA ratios in the field, the values obtained in this study are comparable with other studies of fish larvae on Atlantic cod and haddock (Buckley and Lough 1987).

The early skewed-right RNA–DNA ratio distributions and wide range (Fig. 5) suggest, due to the extended hatching of the cohort of larval herring, that the observed protein growth rates may partly be caused by larvae of slightly different ages. It may also be a function of a few larvae learning to feed and supplementing the energy from their yolk sac reserve. As the mode moves right from Calendar Day 90 to 124, the percentage in the low RNA–DNA ratio categories reduce. From Calendar Day 94 on, there is a suggestion of bimodality in the distributions which could indicate that a group has learned to feed well and has a head start on the rest of the cohort. More detailed samples are needed to determine whether or not this apparent feature is real. As the mode progresses to the right over time, the RNA–DNA ratio distribution becomes more truncated and is similar to that found for field-caught populations of sand lance and haddock (Buckley 1984; Buckley and Lough 1987). The distribution on Calendar Day 124 + 126 shows that there are no larvae below the critical ratio of about 2, which means that they have either been lost (due to predation or starvation) or their feeding abilities have increased so they can easily meet their basic nutritional requirements. The truncated distribution above an RNA–DNA ratio of 8 may reflect net evasion by the larger, more actively growing animals, or it may be that the larvae have reached a maximal protein growth rate. We feel that net evasion is a more likely explanation.

To the best of our knowledge, this study is the first to look at the RNA–DNA ratios of individual fish larvae smaller than 800 μg dry weight. This increased sensitivity was gained by using a fluorometric technique for analysis of the nucleic acids. We feel that the speed, simplicity, and versatility of this technique for measuring growth and condition in larval fish emphasize the usefulness of incorporating the technique into sampling designs for other larval fish studies.

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Copper Intoxication in Chinook Salmon (*Oncorhynchus tshawytscha*) Induced by Natural Springwater: Effects on Gill Na^+, K^+-ATPase, Hematocrit, and Plasma Glucose

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Beckman, B. R., and W. S. Zaugg. 1988. Copper intoxication in chinook salmon (*Oncorhynchus tshawytscha*) induced by natural springwater: effects on gill Na^+, K^+-ATPase, hematocrit, and plasma glucose. Can. J. Fish. Aquat. Sci. 45: 1430–1435.

Chinook salmon (*Oncorhynchus tshawytscha*) parr and smolts were exposed to natural springwater with an elevated copper concentration. Gill Na^+, K^+-ATPase activity in parr was unaffected by 18 h of exposure, while significant inhibition was found in smolts. Under the same exposure conditions, significant increases in hematocrit and plasma glucose were found in both parr and smolts. The results suggest that only the Na^+, K^+-ATPase enzyme associated with chloride cells in the gill of smolts is susceptible to inhibition by copper, thus explaining the lack of enzyme inhibition found in parr.

Des tacons et des smolts de saumon quinnat (*Oncorhynchus tshawytscha*) ont été exposés à de l'eau de source naturelle ayant une teneur élevée en cuivre. L'activité de l'ATPase-Na^+, K^+ dans les branchies des tacons n'a pas été touchée par 18 h d'exposition bien qu'on ait observé une inhibition importante chez les smolts. Dans les mêmes conditions d'exposition, on a observé une hausse significative de l'hématocrite et du glucose du plasma tant chez les tacons que les chez les smolts. Les résultats donnent à penser que seulement l'enzyme ATPase-Na^+, K^+ associée aux cellules de chlore dans les branchies des smolts est susceptible d'être inhibée par le cuivre, ce qui explique l'absence d'inhibition de l'enzyme observée chez les tacons.

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Several studies have demonstrated that aqueous solutions of copper can be toxic to salmonids (Brown et al. 1974; Buckley et al. 1984). Increases in alkalinity or hardness of the aquatic media can attenuate the effect of a given copper concentration (Howarth and Sprague 1978; Miller and Mackay 1980). Many salmonids inhabit environments in which concentrations of carbonate and calcium are low; therefore, even small amounts of dissolved copper can be toxic. Fish exposed to copper show decreased feeding activity, reduced growth, and accumulations of copper in body tissues (Buckley et al. 1982). Copper also disrupts osmoregulatory function (Lauren and McDonald 1986) and inhibits the activity of gill Na^+, K^+-ATPase (Lorz and McPherson 1976; Stagg and Shuttleworth 1982; Lauren and McDonald 1987), an enzyme involved in the osmoregulatory process. Fish have also shown a stress response with increased blood corticosteroid levels upon exposure to copper (Donaldson and Dye 1975; Schreck and Lorz 1978).

Lorz and McPherson (1976) reported a differential response to copper by juvenile coho salmon (*Oncorhynchus kisutch*) progressing through a smolting cycle. Smoltification involves a host of physiological, morphological, and behavioral changes resulting in the transformation of a river-dwelling parr into an actively seaward migrating smolt (Hoar 1976; Folmar and Dickhoff 1980; Wedemeyer et al. 1980). These changes may not occur synchronously, leading to some confusion as to what constitutes a smolt. For the purposes of this article, a smolt was considered to be a juvenile salmon that had obtained a silvery integument and had developed gill Na^+, K^+-ATPase activities significantly greater than parr.

In our laboratory, it was observed that untreated springwater produced a toxic effect on chinook salmon (*O. tshawytscha*) smolts. Subsequent analysis showed that copper was present in this water in elevated concentrations. Experiments were conducted to explore the effects of acute copper exposure on gill Na^+, K^+-ATPase activity, hematocrit, and plasma glucose concentration in both parr and smolts of chinook salmon. As significant responses were elicited in a short time, short-term experiments were conducted.

Materials and Methods

Experiments were conducted in 1986 with chinook salmon parr (August and September) and smolts (May and August). Parr were obtained from production raceways of the Little White Salmon National Fish Hatchery (Cook, WA) and transported to the National Marine Fisheries Service's Cook Field Station (Cook, WA). Fish were held for 4 d in August and 15 d in September before experiments were begun. Holding was in 1.5-m-diameter concrete tanks with continuous water flow from the Little White Salmon River. Fish ranged from 9 to 20 g and were

TABLE 1. Summary of experimental procedures, showing experimental groups, date of experiments, water sources to which groups were exposed (baseline represents fish not exposed to experimental manipulations), time fish were exposed to water source (hours), and experimental parameters measured (A = gill Na^+, K^+ − ATPase, G = plasma glucose concentration, H = hematocrit).

Experimental group	Date	Water source	Exposure period	Parameters measured
LWS Parr	Aug. 25		Baseline	A, G, H
		Spring	18	A, G, H
LWS Parr	Sept. 19		Baseline	A, G, H
		Spring	18	A, G, H
		River	18	A, G, H
Ab Smolts	May 19		Baseline	A, G, H
		Spring	4, 24, 48	A, G, H
McN Smolts	Aug. 3		Baseline	A, G, H
		Spring	4, 18	A, G, H
		Spring/river	18/48, 18/72	A
	Aug. 12	Spring	18	A, H
		River	18	A, H

not fed after arrival at Cook to eliminate accumulation of fecal materials in experimental containers. For the May experiments, smolts were collected from a production raceway of the Abernathy Salmon Culture and Technology Center (U.S. Fish and Wildlife Service, Longview, WA). Fish weighed from 11 to 40 g and were held for 6 d prior to initiation of experiments under conditions similar to the parr. For experiments in August, migrating fall chinook salmon smolts were collected from the fish bypass system at McNary Dam (Columbia River, River Kilometre 470). These fish originated from a variety of hatchery and wild stocks and ranged from 8 to 22 g. They were held at Cook for 3 or 12 d before experiments were begun.

Experiments were conducted in 25-L brown plastic buckets set in a Living Stream Aquarium (Frigid Units Inc. Toledo, OH) to maintain a 10°C temperature. Eight (May) or 10 (remainder of experiments) fish were introduced into an experimental container holding 20 L of springwater (48 μg copper/L, hardness 13.3 mg/L as $CaCO_3$, alkalinity 21.0 mg/L as $CaCO_3$, and pH 6.3). On two occasions (Table 1), fish were exposed to 20 L of water from the Little White Salmon River as a control (3 μg copper/L, hardness 14 mg/L as $CaCO_3$, alkalinity 19 mg/L as $CaCO_3$, and pH 7.7). Air from airstones was continuously bubbled through the water, and the buckets were covered with plastic to prevent fish from jumping out. All the fish in a given bucket were removed and processed after 4, 18, 24, or 48 h (Table 1). Baseline values of experimental parameters for each group were established by immediately processing fish removed from the concrete holding tanks on the day of an experiment. In 48-h exposures, all the water in a bucket was replaced after the first 24 h. In the August 3 experiment, 20 additional fish were first exposed to springwater for 18 h and then allowed to recover for either 48 or 72 h in river water before they were processed.

At the end of an experimental period, fish were stunned with a blow to the head and the caudal peduncle was severed. Blood was collected in heparinized microhematocrit tubes and centrifuged at 1200 g for 3 min. The collected plasma was stored at −25°C until analyzed. Plasma glucose values were determined spectrophotometrically using reagents supplied by Sigma Chemical Co. (St. Louis, MO, procedure No. 315, 1985). Gill filaments were cut from arches and stored at −25°C in 1 mL of a solution containing 0.3 mol sucrose/L, 0.02 mol EDTA/L, and 0.1 mol imidazole/L (pH 7.1) until determination of gill Na^+, K^+-ATPase activity using a slight modification of the method of Zaugg (1982).

Statistical comparisons were made with a one-way ANOVA followed by Tukey's comparison of means. Differences were considered to be significant at $p < 0.05$. Results from experiments on different dates were not compared statistically because fish were from different stocks and had not been collected and held similarly.

Results

Hematocrits of Little White Salmon parr were significantly greater than baseline values after an 18-h exposure to springwater on both August 21 and September 19 (Fig. 1). Parr treated as controls (river water exposure) on September 19 displayed no change. Hematocrits of smolts from Abernathy increased significantly after a 4-h exposure to springwater; a further significant increase was found after 24 h. Hematocrits of migrant smolts from McNary Dam showed a nonsignificant increase after 4 h and a significant increase after 18 h. Controls from McNary (river water) had hematocrits which were significantly lower than fish exposed 18 h to springwater. Baseline values of hematocrit in parr were lower than those found in smolts.

Plasma glucose levels of parr exposed to springwater for 18 h were significantly higher than baseline (Fig. 2). Parr treated as controls had elevated glucose levels that were not significantly higher than the baseline. Glucose levels of Abernathy smolts were not affected by a 4-h exposure to springwater, but there was a significant increase after 24 h and a further significant increase after 48 h. Smolts from McNary Dam had elevated glucose levels after 18 h, but values were not significantly different from baseline. The lack of statistical difference was likely due to the large standard error associated with these measurements. Baseline levels of plasma glucose found in parr were lower than those found in smolts.

There were no significant changes in gill Na^+, K^+-ATPase activities of Little White Salmon parr under any treatment (Fig. 3). Enzyme activities of Abernathy smolts declined significantly after a 24-h exposure to springwater and there was a further nonsignificant decrease after 48 h. Smolts from McNary Dam showed a significant decrease in enzyme activity after only a 4-h exposure, with no further significant decrease after 18 h. The activities of smolts allowed to recover for either 48 or 72 h in river water following an 18-h springwater exposure

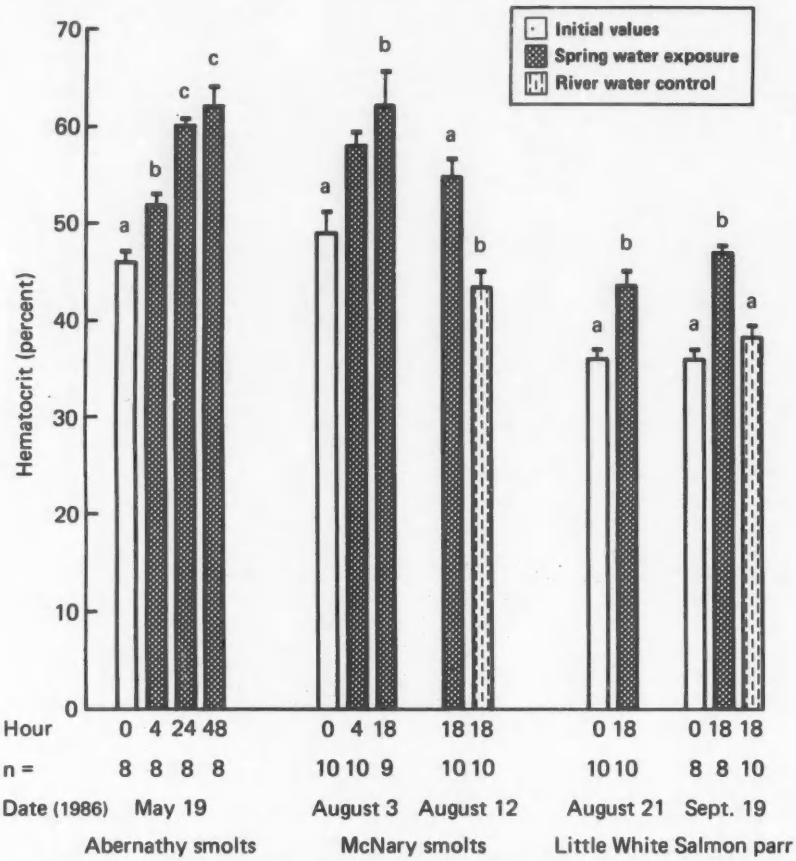
Initial values
Spring water exposure
River water control
Hematocrit (percent)
70
60
50
40
30
20
10
0
Hour 0 4 24 48 0 4 18 18 18 0 18 0 18 18
n = 8 8 8 8 10 10 9 10 10 10 10 8 8 10
Date (1986) May 19 August 3 August 12 August 21 Sept. 19
Abernathy smolts McNary smolts Little White Salmon parr

FIG. 1. Effect of springwater exposure on hematocrit of chinook salmon parr and smolts. Each bar represents an average + SE. Bars marked "b" are significantly different from bars marked "a"; bars marked "c" are significantly different from those marked "b" ($p < 0.05$, Tukey's multiple comparison test).

were significantly lower than the 18-h exposure. On August 12, an 18-h exposure of McNary Dam smolts to springwater resulted in gill Na^+, K^+-ATPase activities that were significantly less than those of control fish in river water. The baseline activities found in parr were much lower than those found in either group of smolts.

Discussion

Several workers have shown that increased hematocrits result from exposure of salmonids to copper (McKim et al. 1970; Waiwood 1980; Buckley et al. 1984). This occurred in both chinook salmon parr and smolts in our study. Zaugg and McLain (1986) showed a seasonal increase in hematocrit associated with smoltification in coho salmon. Thus, the differences in initial value of hematocrits that we found between parr and smolts may be the result of the smoltification process. Our study indicated the rapidity with which hematocrits can increase — significant changes were found within 4 h, suggesting that the effects of copper intoxication begin within this short time period. Waiwood (1980) presented evidence to show that increased hematocrits were associated with blood concentration and not red blood cell swelling. Results from our laboratory (unpublished) show increases in plasma protein and hemoglobin concentrations associated with the rise in hematocrits, supporting Waiwood's (1980) results.

Donaldson and Dye (1975) and Schreck and Lorz (1978) showed that acute copper exposure induces increased blood cortisol levels in salmon. This is considered a primary response to stress whereas an increased level of blood glucose is designated as a secondary response (Mazeaud et al. 1977). The elevated plasma glucose levels found in our study would indicate that exposure to copper effected a stress response in both parr and smolts. The relative level of stress is difficult to judge, however, due to differing acclimation periods of test groups. It is possible that some fish did not completely recover from the stress of transport prior to the start of an experiment, thereby altering their capacity to respond to copper exposure, resulting in a greater stress response than would have occurred in fish allowed a long-term acclimation. Initial plasma glucose levels in smolts were elevated compared with parr. This could have been caused by the smoltification process itself, by an incomplete recovery of the smolts from the stress of transportation, by an incomplete adaptation to the holding environment, or perhaps by a greater susceptibility to stress in smolts relative to parr. Parr treated as

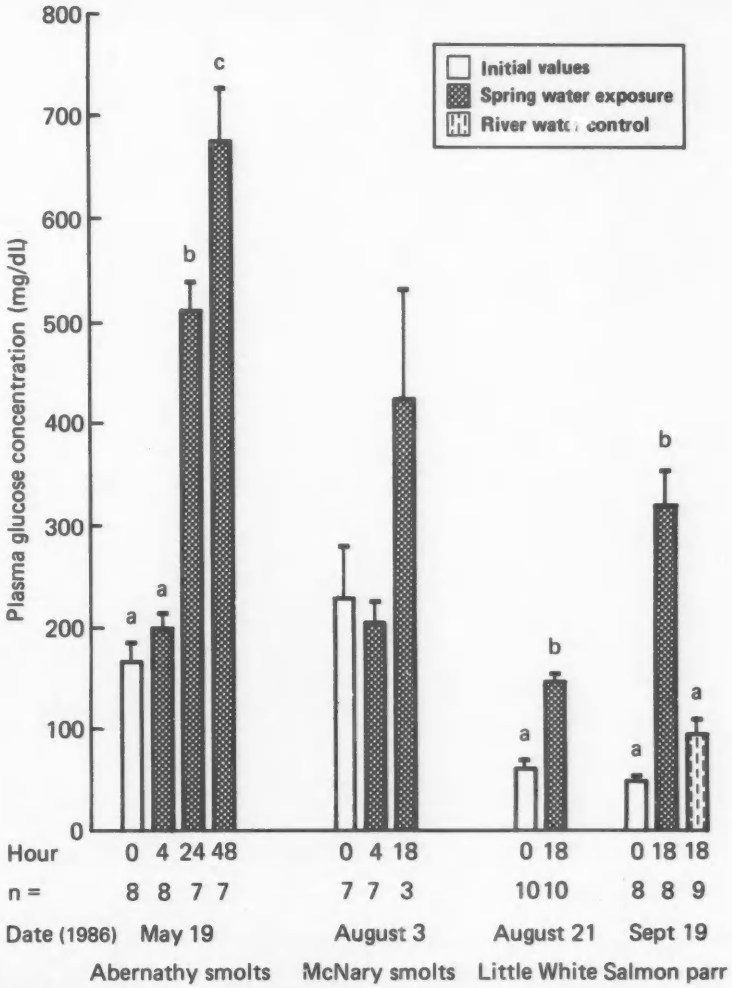

FIG. 2. Effect of springwater exposure on plasma glucose of chinook salmon parr and smolts. Each bar represents an average + SE. Bars marked "b" are significantly different from those marked "a"; bars marked "c" are significantly different from those marked "b" ($p < 0.05$, Tukey's multiple comparison test).

controls showed an increased glucose concentration, which would suggest that there was some stress associated with experimental conditions. However, the significantly higher glucose levels found in parr exposed to water containing copper show that copper created an additional stress on the fish.

Gill Na^+, K^+-ATPase activity of chinook salmon smolts was very sensitive to copper exposure; declines in activity of greater than 50% were found within 24 h. Lorz and McPherson (1976) found declines in enzyme activities for coho salmon smolts of 35–80% with 56–144 h of exposure to 30 μg copper/L, while Lauren and McDonald (1987) found a 33% decrease in activity of juvenile rainbow trout exposed to 55 μg copper/L for 24 h. Stagg and Shuttleworth (1982) exposed seawater-adapted flounder (*Platichthys flesus*) to copper for 30 d and reported some evidence of enzyme inhibition. Environmental copper inhibits gill Na^+, K^+-ATPase activity, and as our results show, this can happen in time periods of less than 24 h. Since we did not find any restoration of activity within a 2- to 3-d recovery period, it appears that copper inactivation of the enzyme persists for at least several days.

Gill Na^+, K^+-ATPase activity of parr was not inhibited when fish were exposed to springwater. In contrast, when the enzyme from parr gill was exposed to copper in vitro, we observed a clear inhibtion of activity (unpublished). The enzyme from smolt gill was not only inhibited by copper in vitro but also by exposure of the gill in vivo. Therefore, there appears to be a difference between parr and smolt gill Na^+, K^+-ATPase based upon in vivo accessibility to copper ions.

Two different functions and locations in the gill for Na^+, K^+-ATPase have been proposed. In seawater-adapted fish, a concentration of Na^+, K^+-ATPase has been found in the chloride cell and its activity has been associated with monovalent ion extrusion. Investigators have shown that, in both freshwater- and seawater-adapted fish, there is an additional Na^+, K^+-ATPase enzyme involved in NH_4^+/Na^+ exchange localized in respiratory cells (Epstein et al. 1980; Girard and Payan 1980;

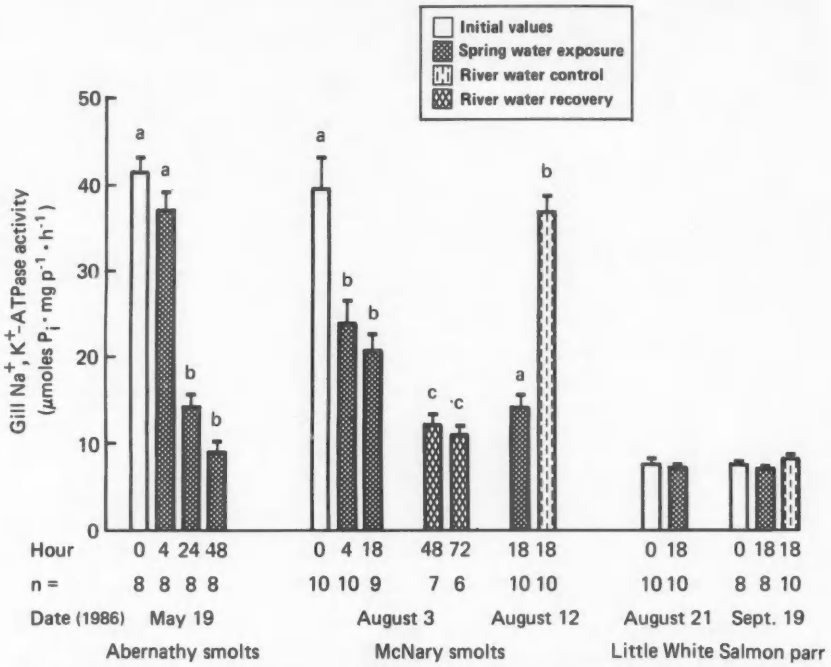

FIG. 3. Effect of springwater exposure on gill Na^+, K^+-ATPase activity of chinook salmon parr and smolts. Each bar represents an average + SE. Bars marked "b" are significantly different from those marked "a"; bars marked "c" are significantly different from those marked "b" ($p < 0.05$, Tukey's multiple comparison test).

Evans et al. 1982; Foskett et al. 1983). Observable damage to chloride cells was found when fish were exposed to copper and other heavy metals (Baker 1969; Mallatt 1985). In neither our study nor that of Lorz and McPherson (1976) was the inhibition of gill Na^+, K^+-ATPase complete in smolts: the residual levels of activity were similar to that of parr. It would seem then that the exposure of smolts to copper results in the inhibition of gill Na^+, K^+-ATPase activity associated with the chloride cell. Similarly, this form of gill Na^+, K^+-ATPase has been shown to be sensitive to actinomycin-D whereas that found in freshwater-adapted fish appears to be insensitive (Motais 1970; Gallis et al. 1979). Thus, two forms of gill Na^+, K^+-ATPase may exist and may be located differentially in the gill, providing an explanation for the differential sensitivity of parr and smolt gill enzyme to environmental copper.

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Littoral Zoobenthic Biomass in Lakes, and Its Relationship to Physical, Chemical, and Trophic Factors[1]

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Rasmussen, J. B. 1988. Littoral zoobenthic biomass in lakes, and its relationship to physical, chemical, and trophic factors. Can. J. Fish. Aquat. Sci. 45: 1436–1447.

The hypothesis that littoral morphometry exerts a strong effect on zoobenthic biomass was tested in Lake Memphremagog, Quebec–Vermont, by regression analysis of the relationship between littoral zoobenthic biomass and littoral slope, exposure (Lake area visible from sampling site), and trophic status (micrograms of chlorophyll a per litre). Eighty-one percent of the variance in Log littoral zoobenthic biomass (LZB) could be explained by

$$\text{Log LZB} = 1.3069 + 0.6587 \text{ Log Chlorophyll } (\mu g/L) - 0.0795 \text{ Exposure} \times \text{Slope } [(km^2)^{0.25} \times (\%)^{0.5}].$$

When the predictions from this relationship were compared with littoral zoobenthos estimates from other lakes, residuals were positively correlated with the calcium concentration of the water ($r = 0.41$) and negatively correlated with the chloride concentration ($r = -0.51$). The regression equation obtained for all of the sites was

$$\text{Log LZB} = 1.0940 + 0.3511 \text{ Log Chlorophyll} - 0.1632 \text{ Log Slope} - 0.0518 \text{ Exposure} \times \text{Slope} + 0.4416 \text{ Log Calcium (mg/L)} - 0.2822 \text{ Log Chloride (mg/L)}$$

and explained 80% of the variation in Log LZB. Sites situated within deltas from stream inflows did not differ significantly from these that did not (ANCOVA), indicating that coarse allochthonous input from these fluvial sources was not a major factor determining littoral zoobenthic biomass in these lakes.

L'hypothèse selon laquelle la morphométrie du littoral exercerait un effet notable sur la biomasse zoobenthique a été mise à l'essai dans le lac Memphrémagog (Québec–Vermont) par une analyse de régression de la relation entre la biomasse zoobenthique littorale et la pente du littoral, l'exposition (superficie du lac visible du point d'échantillonnage) et l'état trophique (microgrammes de chlorophylle a par litre). Quatre-vingt-un pour cent de la variance du logarithme de la biomasse zoobenthique littorale (LZB) pouvait s'expliquer par la formule suivante :

$$\log \text{LZB} = 1{,}3069 + 0{,}6587 \log \text{Chlorophylle } (\mu g/L) - 0{,}0795 \text{ Exposition} \times \text{Pente } [(km^2)^{0.25} \times (\%)^{0.5}].$$

Lorsqu'on a comparé les prédictions tirées de cette relation aux estimations du zoobenthos littoral pour d'autres lacs, les résidus présentaient une corrélation positive avec la concentration de calcium dans l'eau ($r = 0{,}41$) et négative avec la concentration de chlore ($r = -0{,}51$). L'équation de régression obtenue pour tous les sites était la suivante :

$$\log \text{LZB} = 1{,}0940 + 0{,}3511 \log \text{Chlorophylle} - 0{,}1632 \log \text{Pente} - 0{,}0518 \text{ Exposition} \times \text{Pente} + 0{,}4416 \log \text{Calcium (mg/L)} - 0{,}2822 \log \text{Chlore (mg/L)}$$

et expliquait 80 % de la variation de log LZB. Les sites qui se trouvaient dans les deltas des cours d'eau qui se jetaient dans le lac ne différaient pas de façon significative des autres (ANCOVA), ce qui indique que les matériaux grossiers d'origine allogène provenant de ces sources fluviales ne constituent pas un facteur déterminant pour la biomasse zoobenthique littorale de ces lacs.

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The zoobenthos are animals that live in association with a substrate–water interface (Hutchinson 1967). In the littoral zone, the substrate used by benthic invertebrates can be either the sediment (epifauna and infauna) or the vegetation (epiphytic invertebrates). Since the partitioning of the fauna between these two substrates is highly variable spatially and temporally, I will treat the littoral zoobenthos (LZB) as a single vertically integrated community and quantify its abundance as biomass per square metre. These animals utilize particulate organic matter that settles to, or grows upon, the substrate, and they serve as a major trophic base for fish communities (Efford 1972; Eggars et al. 1978; Jónasson 1978; Wissmar and Wetzel 1978). Thus, zoobenthic biomass has been considered by many limnologists to be an index of the potential productivity of a lake for fish (Northcote and Larkin 1956; Rawson 1958, 1960). This has resulted in the development of models that predict fish biomass and yield from zoomacrobenthic biomass (Hanson and Leggett 1982; Matuszek 1978).

The littoral (which may support rooted macrophytes) has the highest rates of primary and secondary production (Winberg 1972; Kajak et al. 1972; Wetzel and Allen 1972) and the highest diversity, biomass, and turnover of benthos (Brinkhurst 1974; Johnson 1974) and fish (Werner et al. 1977; Keast et al. 1978; Wissmar and Wetzel 1978). It is the part of the lake that receives the greatest input of detrital material from the watershed (Wetzel

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and Allen 1972). Despite its disproportionate importance, the spatial, temporal, and three-dimensional heterogeneity of the littoral zone has limited our understanding of littoral processes.

Rasmussen and Kalff (1987), in their analysis of literature data, found that the best single predictor of littoral zoobenthic biomass was phytoplankton biomass (micrograms of chlorophyll *a* per litre); however, their best multiple regression model explained <50% of the variance, despite significant contributions from chlorophyll *a* and lake area, mean littoral slope, and water colour. Two major factors probably contributed to this large residual variation. The first is the poor sampling efficiency of most remote-operated grabs and weed samplers in the littoral (Downing and Cyr 1985). The second is the high degree of local heterogeneity in exposure and littoral slope, and the imprecision that results from using whole-lake rather than site-specific measures for some predictors. Furthermore, input of coarse allochthonous detritus from the watershed may contribute substantially to the LZB and this factor was not considered in the previous analysis.

The purpose of this study was to develop better empirical models for LZB by using improved sampling methodology, together with site-specific measurements of littoral slope, exposure to waves, and sediment characteristics as predictors. In an attempt to evaluate the possibility that allochthonous detritus is an important contributing factor, sites located within stream deltas were included in the analysis and compared with sites distant from stream inflows. Other predictors tested were lake trophic status (chlorophyll and transparency), water chemistry parameters (hardness, alkalinity, pH, and the concentrations of major ions), and water colour. I first examined the variation in LZB within Lake Memphremagog (Quebec–Vermont) which is highly variable in its littoral morphometry (Duarte and Kalff 1986) and has an inflow–outflow gradient in phytoplankton biomass and nutrient concentrations (Ross and Kalff 1975; Gascon and Leggett 1977). Relationships obtained from this lake were then compared with data obtained from littoral stations in a number of other lakes in eastern Quebec (Table 1) and from the literature.

Methods

Half of the sites sampled ($n = 29$) were situated in the dimictic and oligo- to mesotrophic Lake Memphremagog (Quebec–Vermont), which varies greatly in littoral morphometry and wind exposure along its 45-km length (Duarte and Kalff 1986). An additional 28 sites from 11 smaller lakes were also sampled. These lakes (Table 1) ranged from oligotrophic to strongly eutrophic and also presented a broad range of littoral slopes and exposures. The sites sampled were chosen with the aim of maximizing the range of slope, exposure, and phytoplankton biomass (milligrams of chlorophyll *a* per cubic metre) and also the range of combinations between the variables, in order to minimize collinearity. At each site, slope was measured by echosounding along a transect perpendicular to shore, as described by Duarte and Kalff (1986). The benthos and submerged macrophyte biomass were sampled at the point of maximum plant canopy height on each littoral transect. The index of exposure was calculated from 1:25 000 maps as the contour (circular) integral of the fetch ($\S Fd\theta$, where F = fetch or distance to nearest shoreline (Håkanson 1981) for a given angle θ). This circular integral describes the area exposed (or visible) from the sample site. Sediment water content and organic matter content were also measured at each site (Duarte and Kalff 1986). Secchi disk transparency, concentration of chlorophyll *a* (milligrams per cubic metre), water colour, and conductivity (microsiemens, 25°C) were measured beyond the macrophyte bed at each site, since it was intended that they characterize the water moving into the littoral through wave action. Moreover, it is impractical to measure transparency and chlorophyll concentration within weed beds, where the slightest disturbance of the plants will greatly alter the measurements. Plankton and nutrient concentrations, plus water transparency within weed beds, can be inhibited or otherwise influenced by submerged macrophytes in a variety of ways (Goulder 1969; Brammer 1979; Wetzel 1983; Carpenter and Lodge 1986), and these effects might often extend beyond the macrophyte bed. Thus the measures taken adjacent to the littoral may not be completely independent of the influence of macrophytes. The sites of Lake Memphremagog were sampled monthly during August–October 1984 and May–August 1985. The sites in the other Quebec lakes were sampled monthly during May–October 1985.

Biomass of epiphytic invertebrates (grams per kilogram of macrophytes) was estimated using a slightly modified version (Fig. 1) of the sampler described by Downing (1986). A SCUBA diver carefully enclosed a sample of undisturbed vegetation and trimmed off all protruding plants. This sampler trapped even mobile animals such as amphipods without disruption. As the sampler was removed from the water, the water inside was allowed to drain through the 0.2-mm screen on the side, and the contents of the sampler were bagged. In the laboratory, the vegetation was carefully rinsed free of animals, marl, and most periphyton, indentified to species, spun for 1 min in a salad spin-dryer, and weighed. The remaining material in the sample, including the animals, was then preserved in 5% formalin. On each sampling date, when vegetation was present, two such samples were taken in the middle of the canopy, two within 50 cm of the substrate, and two within 1 m of the surface, if the canopy grew that high. The total biomass of submerged macrophytes was then estimated by taking five replicate 0.1-m^2 quadrat samples that were randomized by tossing the quadrat from a boat within the area of maximum canopy height. The SCUBA diver, at the same time, took five 40-cm^2 cores of sediment, and these were screened through a 0.2-mm screen, and the sieve residue, containing the animals, was preserved in 5% formalin. Animals were picked from the samples under a dissecting microscope and organisms were blotted and weighed to the nearest 0.1 mg.

Biomass of epiphytic invertebrates (grams per kilogram of plant) was multiplied by total biomass of submerged macrophytes (kilograms per square metre) to yield grams per square metre, and this product was added to the biomass estimated for the sediment benthos to yield total zoobenthos (grams per square metre). Values estimated by quadrats and cores were corrected to represent grams per square metre of lake surface by multiplying the area sampled by cos α where α is the slope of the bottom in degrees. The six estimates obtained for each site (one for each month of the ice-free season) were averaged, and these averages were entered as the dependent variable (LZB) in the regression analyses. The analysis included site-specific estimates of LZB from the literature, when they either included epiphytic invertebrates or were obtained from sites with no weed canopy, and when they could be associated with morphometric, trophic, and chemical data.

Linear regression was used to model the relationships between variables (Draper and Smith 1981). For the Lake Memphremagog analysis, Log LZB was regressed against Log

TABLE 1. Lakes included in the analysis, limnological features, and littoral zoobenthic biomass. Blank entries are repetitions of the entry above.

Lake	Lat. (N)	Long. (W)	Slope (%)	Exposure (km^2)	Cond. (μS)	Ca (mg/L)	Cl (mg/L)	Colour (mg Pt/L)	Chl *a* (g/L)	Secchi (m)	LZB (g/m^2 wet wt.)	Sources of data[a]
Memphremagog, Quebec	45°14′	72°14′										
Newport Bay	44°55′	72°10′	0.2	3.1	145	18	5.2	8	12	2.5	118.5	1, 2, 3, 4
			1.0	5.8					10.4	3	66.0	
			2.0	4.1						2.9	67.3	
South Basin	45°00′	72°14′	20	22.5	143	18.5	5.5	6	6.2	3.5	15.8	
			1.1	1.95					6.0	3.8	40.0	
			1.7	12.3				5		3.3	34.9	
			18	14.5					6.1		11.2	
			1.5	16.2				5	6.8	3.4	53.4	
			4.0	35.8				6	6.0	3.6	26.0	
			18.0	17.2				3	5.5	3.8	11.3	
			5	20.6				5	6.2		37.3	
Fitch Bay	45°05′	72°12′	14	6.7	132	15.5	4.6		3.5	3.8	29.0	
			2.1	2.62				6	3.2	3.6	35.4	
			3.8	3.7					3.6		37.0	
Central Basin	45°07′	72°17′	7.0	19.7	141	17.3	5.1	2	4.2	3.8	10.6	
			11	24.4	143			3	4.5	4.3	12.9	
			0.9	3.83				2	3.5	4.2	36.1	
			0.2	4.8				4	4.5		47.2	
			6.6	12.8				2	3.5	4.8	25.5	
			1.8	2.1	140				3.0	5.0	43.3	
			19	2.0							11.3	
			5.4	16.9	143				3.4	4.9	18.3	
North Basin	45°12′	75°13′	1.5	10.8	142	18	5.9		3.1		22.0	
			4.2	3.2					3.0		27.8	
			3.5	11.9						5.3	17.5	
			8.0	10.9					3.1	5	14.6	
			0.4	5.1					4.1	5.1	52.2	
			3.0	6.2					3.2	5.2	15.2	
			2.0	7.4					3.0	5.3	25.1	
Brompton, Quebec	45°26′	72°09′	3.0	2.2	62	4.1	1.9	19	1.3	4.2	19.5	1, 5
			7.0	11.2							15.4	
Brome, Quebec	45°15′	72°30′	0.6	6.5	104	9.3	13	4	10.1	2.8	36.7	1, 5
			5.0	13.6							19.9	
D'argent, Quebec	45°18′	72°18′	18	0.89	94	7.6	12.1	32	3.5	2.6	9.3	1, 5
			6.0	0.87							14.7	
			1.0								20.3	
Hertel, Quebec	45°32′	73°09′	5.1	0.29	86	12	2	5	3.0	3.5	42.5	1, 8
			11								18.4	
			5.0								36.1	
Magog, Quebec	45°15′	72°10′	0.5	1.8	165	15.4	6.4	11	11	2.3	62.6	1, 5
			5.2	10.1							26.5	
			9.0								24.1	
Orford, Quebec	45°18′	72°16′	1.8	0.9	265	7	41	0	1.6	7.1	7.4	1, 5
			4.2	0.7							6.1	
			15	0.3							4.2	
			14	1.2							4.5	
Roxton Pond, Quebec	45°28′	72°39′	0.8	1.7	120	9.4	4.2	32	8.5	2.3	21.8	1, 5
Silver, Quebec	45°38′	72°48′	12	0.67	110	9	1.8	0	3.5	5.8	18.7	1, 6, 7
			2.2								20.1	
			14	0.45							10.2	
			25	0.67							7.5	
Waterloo, Quebec	45°20′	72°05′	8.6	1.3	148	13.8	23	15	24	0.9	42.8	1, 5, 7
			0.9								36.8	
			0.4	0.09							43.4	
Drolet, Quebec	45°43′	70°53′	5.0	2.1	47	4	2.8	6	4.0	3.8	19.1	1
			0.5								27.1	

TABLE 1. (*Concluded*)

Lake	Lat. (N)	Long. (W)	Slope (%)	Exposure (km²)	Cond. (μS)	Ca (mg/L)	Cl (mg/L)	Colour (mg Pt/L)	Chl *a* (g/L)	Secchi (m)	LZB (g/m² wet wt.)	Sources of data[a]
Bromont, Quebec	45°16′	72°40′	1.0	0.23	89	10	1	11	9.6	2.1	85.5	1, 4
Great Slave, N.W.T.												
West Basin	61°30′	115°00′	1.5	19400	213	20	8	15	2.5	3.8	3.6	7, 8
Christie Bay	62°30′	111°00′	2.0	450	183	17	7	5	1.9	5	20.1	
Wabamun, Alberta												
Kapasiwin Bay	53°31′	114°26′	0.1	3	390	40	3	5	10	2.8	123.7	11, 12
West Basin			3.5	28							42.4	
Narrow, Alberta	54°35′	113°37′	18.8	0.9	290	35	3	8	3.5		52.3	13, 14, 15, 16
			17								39.4	
			21								19.3	
			26.3	0.23							13.9	
Tjeukemeer, Holland	52°50′	5°40′E	1.0	21	900	32	42	30	27	0.7	26.5	17, 18
Lake Superior												
Batchawana Bay, Ontario	47°00′	88°00′	6.0	35	79	13.2	1.3	7	0.7	9.5	6.3	18, 19, 20
Mirror, New Hampshire	43°56′	71°45′	10	0.15	120	2.4	2.1	5	2	6	5.5	21, 22
Zelenetzkoye, USSR	66°30′	32°20′E	15	0.24		3	17	36	0.3	4.7	3.0	23, 24
Krivoe, USSR	66°00′	32°00′E	10	0.49		4	17	24.4	0.5	4.5	4.0	
Krugloe, USSR	65°00′	32°00′E	1.0	0.24		3	16	125	0.6	1.5	15	

[a]Sources of data: 1, this study; 2, Carlson et al. 1979; 3, R. E. Carlson, unpubl. data; 4, Québec, Service de Qualité des Eaux 1973; 5, Québec, Service de Qualité des Eaux 1978; 6, Québec, Service de Qualité des Eaux 1977; 7, Provencher et al. 1979; 8, Duarte 1987; 9, Rawson 1953; 10, Fee et al. 1985; 11, Noton 1975; 12, J. B. Rasmussen, unpubl. benthos and chemistry data; 13, J. M. Hanson, unpubl. benthos data; 14, Prepas and Trew 1983; 15, Prepas and Vickery 1984; 16, E. E. Prepas, unpubl. chemistry data; 17, Beattie et al. 1972; 18, Dermott 1984; 19, Janus and Vollenweider 1981; 20, Beeton 1969; 21, Walter 1985; 22, Likens 1985; 23, Winberg et al. 1973; 24, Alimov et al. 1972.

Slope, Log Exposure, E × S (Exposure$^{0.25}$ × Slope$^{0.5}$), and Log Chlorophyll. In addition, transparency was tested in place of Log Chlorophyll, and plant biomass and sediment water content were individually tested in place of the set of physical factors (Log Slope, Log Exposure, and E × S). Thus, variable combinations that produced serious collinearities (Belsley et al. 1980; Wilkinson 1986) were avoided. Also, Sediment Water Content (percent) was regressed against Log Slope, Log Exposure, and E × S. For the multilake data set, Log LZB was regressed against Log Slope, Log Exposure, E × S, Log Chlorophyll, Log Calcium, Log Chloride, and Colour. In the multilake data set, plant biomass was not as highly collinear with morphometric variables as it was in Lake Memphremagog. Thus, when plant biomass was tested in the multilake regressions, the morphometric variables were not excluded. Sodium was also tested in place of chloride in this model. Similar analyses were also carried out with Log Molluscan LZB, Log non-Molluscan LZB, and Log Sediment LZB as the dependent variables.

LZB was transformed to the logarithm (base 10) because this produced good linear fit between predicted and observed LZB, in addition to homoscedastic normally distributed residuals for all models tested. Independent variables were transformed when necessary (Box and Tidwell 1962) to meet the statistical requirements of linear regression. Transformations and appropriate units for all predictors are given in Tables 4 and 6.

For some variables, sites within the same lake do not differ whereas for others, within-lake variation is as great as between-lake variation. Thus, for variables such as slope and exposure, each site provides a degree of freedom whereas for chemical variables, the number of actual degrees of freedom should be the numbers of different lakes. The probability estimates calculated for each variable in the regression and the correlation analyses (see Tables 3–6) were, therefore, based on the number of degrees of freedom for the variable in question, rather than on the total number of sites from which data were entered.

The explained variance (R^2) was partitioned into component contributions from each predictor variable by path analysis, as outlined by Li (1975) and Sokal and Rohlf (1982). The component coefficients (d^2) from direct paths are b'^2 (b' are standardized partial regression coefficients) and for indirect (collinear) paths between variables i and j are $2b'_i b'_j r_{ij}$ (where r_{ij} is the correlation coefficient between variables i and j). Since the coefficient of multiple determinations (R^2) is the sum of direct and indirect determinations, the magnitude of the difference $R^2 - \Sigma d_i^2$ will reflect the contribution from collinear paths.

Although the dependent variable in all of these analyses was the mean growing-season LZB at d (the depth of maximum canopy height of macrophytes), within-site variation of zoobenthic biomass (with depth) was also investigated at 12 of the sampling sites during July–August 1985. Each of these stations was sampled at three depths within the littoral transect (at d, $0.5d$, and $1.5d$) in order to permit a comparison of within- and between-transect variation in LZB. These three depths provided a reasonable representation of within-transect variation for LZB; $1.5d$ tended to be near the outer margin of macrophyte growth

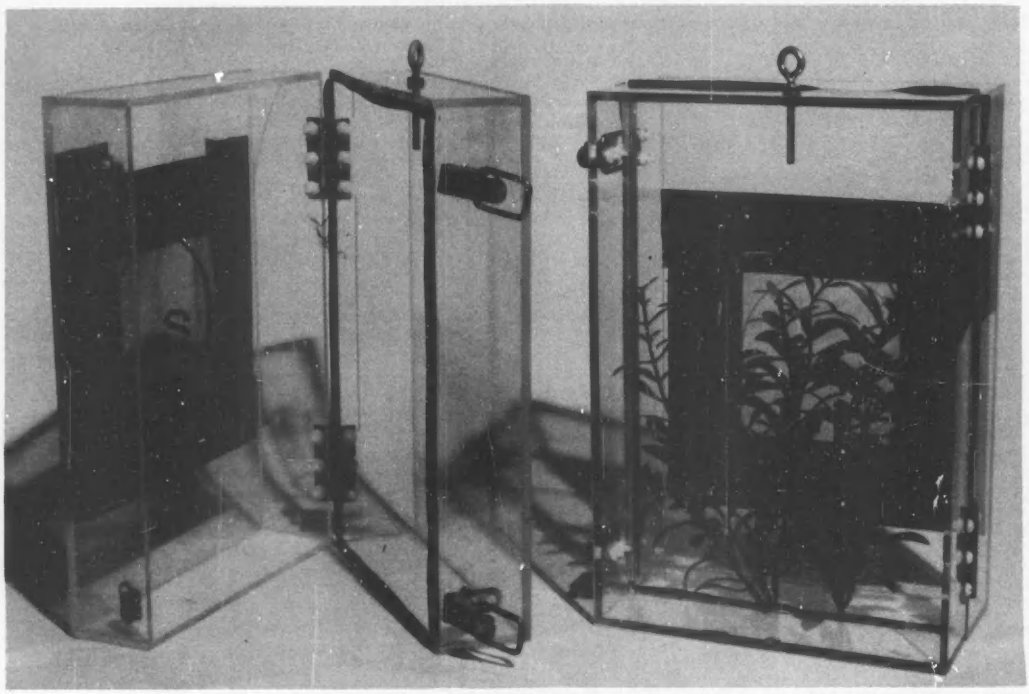

FIG. 1. Box sampler (30 × 20 × 10 cm) used for sampling epiphytic invertebrates. This is a modified version of that used by Downing (1986). S is the side-screen through which water is drained.

TABLE 2. Range and median values for variables measured at 29 stations in (a) Lake Memphremagog and (b) the remaining lakes in Table 1.

	Maximum		Minimum		Median	
Variable	a	b	a	b	a	b
Total zoobenthic biomass (g/m²)	118.5	123.7	10.6	3.3	28.0	24.6
Macrophyte biomass (kg/m²)	2.06	3.05	0.0	0.0	0.47	0.44
Chlorophyll *a* (mg/m³)	12.1	27.0	3.0	0.3	4.1	3.5
Secchi transparency (m)	6.5	9.5	2.9	0.7	4.3	3.8
Slope (%)	20.3	25.0	0.3	0.1	3.5	4.4
Exposure (km²)	35.8	19400	1.95	0.09	7.4	3.0
Sampling depth (m)	4.6	6.0	1.5	1.0	3.0	3.1
Sediment water content (%)	0.84	0.88	0.19	0.18	0.45	0.44

and 0.5*d* was generally near the inner margin of the macrophyte canopy.

Results

The range of variation in total littoral zoobenthic biomass (LZB) was from 3.3 to 123.7 g/m² (Table 1), and the range encountered within Lake Memphremagog was nearly as large (10.6–118.5 g/m²) (Table 2); the between-site standard deviation for Log LZB within Lake Memphremagog was 76% of that obtained for the total data set. LZB includes both the benthos within sediment and on vegetation, and the relative contribution of these two components to the total depended strongly on the biomass of plants (Fig. 2). For the physical variables (slope and exposure), sediment water content, and the plant biomass, the range found within Lake Memphremagog was again nearly as great as that recorded for the whole data set (Table 2). Although chlorophyll *a* concentration and transparency within Lake Memphremagog vary considerably from south to north (Tables 1 and 2), the between-site standard deviation for these variables is only about half of that recorded for the whole data set.

Within Lake Memphremagog, LZB was strongly correlated with these highly variable physical factors, the sediment water content, and plant biomass. The best bivariate correlations are with plant biomass and with littoral slope (Fig. 3; Table 3). These relationships were studied further using regression analysis. Simple regressions using plant biomass and slope as predictors explained 69 and 68% of the variance in Log LZB, respectively Table 4). A multiple regression using slope, chlorophyll concentration, and exposure as predictors explained 81% of this variance (Equation 2), and a multiple regression using plant biomass and chlorophyll concentration explained 83% (Equation 4). Entering Secchi transparency in place of chlorophyll *a* always resulted in a slight drop in explanatory power of the models, but had no other influences on their structure. Equation 3 is shown in Table 4 for comparison with Equation 2, but the remainder of the models containing transparency are not included due to their similarity with those containing chlorophyll as the trophic variable.

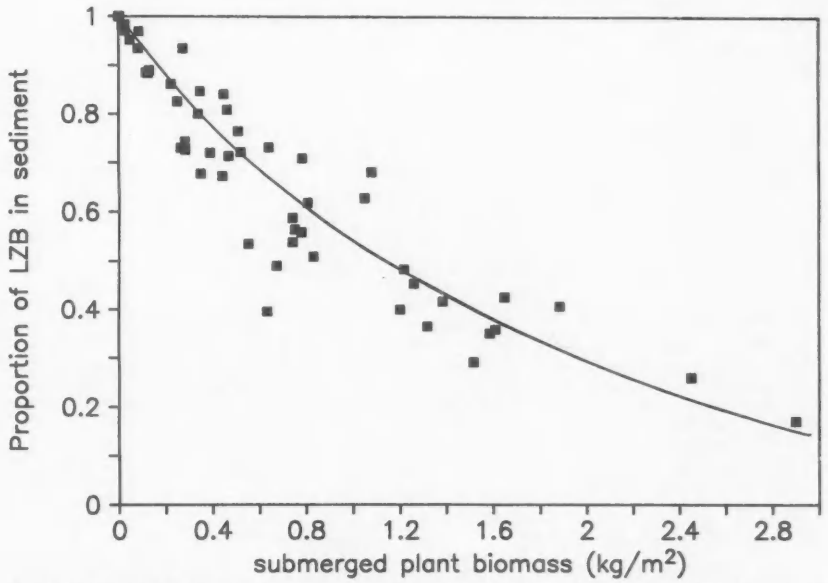

FIG. 2. Relationship between the proportion of litorral zoobenthos (P) sediment and the biomass of submerged macrophytes. The fitted line represents the following equation:

Arcsin ($P^{0.5}$) = 1.572 − 0.7518 plant biomass $(kg/m^2)^{0.4}$, R^2 = 0.91, SE of estimate = 0.082, N = 61.

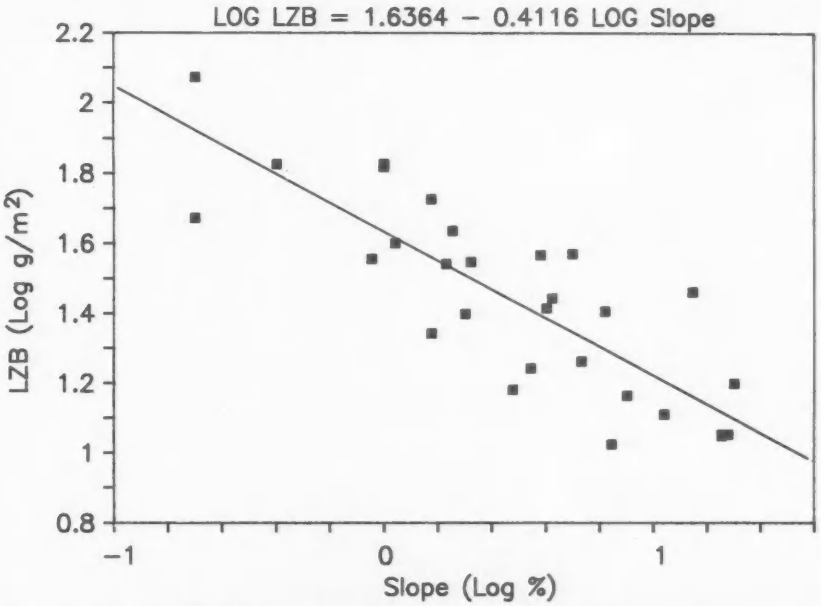

FIG. 3. Relationship between littoral zoobenthic biomass and littoral slope in Lake Memphremagog (R^2 = 0.68, SE of estimate = 0.157, N = 30).

When data from 42 sites in 20 other lakes (Table 1) were included, the correlation between LZB and slope dropped to −0.567 (from −0.822 in Lake Memphremagog; Tables 3 and 4), but the correlations of LZB with plant biomass and chlorophyll did not change significantly ($p < 0.05$). When the multiple regression models developed for Lake Memphremagog were tested using the data from the remainder of the lakes, correspondence between predicted and observed LZB was quite poor (Fig. 4), with a large number of the observations from other lakes falling below the expected values. Although the multilake data set contains a wider range of trophic and humic conditions than Lake Memphremagog, neither chlorophyll,

TABLE 3. Statistically significant ($p < 0.05$) correlation coefficients between the variables measured at the 29 stations in Lake Memphremagog. *Highly significant ($p < 0.01$)

Variable	Slope (Log %)	Exposure (Log km²)	Sampling depth (m)	Chlorophyll *a* (Log mg/m³)	Plant biomass $(kg/m^2)^{0.5}$	Sediment water content (%)
LZB (Log g/m²)	−0.822*	−0.461	n.s.	0.502	0.871*	0.610*
Slope	—	n.s.	0.339	n.s.	−0.881*	−0.734*
Exposure		—	0.433	n.s.	−0.536*	−0.627*
Sampling depth			—	n.s.	n.s.	n.s.
Chlorophyll *a*				—	n.s.	n.s.
Plant biomass					—	0.758*

TABLE 4. Regression models obtained for littoral biomass for 28 sites in Lake Memphremagog. Significance: *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$. The exposure × slope interaction term was transformed as (exposure$^{0.25}$ × slope$^{0.5}$) (units as in Table 1). The original models tested all included Log Slope and Log Exposure plus their interaction. For Equations 2–4, Log Slope and Log Exposure were not significant, and the models were retested without these terms. In Equation 5 the interaction term was not significant.

Equation	R^2	SE of est	$R^2 - \Sigma d^2$	df
(1) Log LZB = 1.627 − 0.401 Log Slope	0.68	0.157		28
(2) Log LZB = 1.267 + 0.711 Log Chlorophyll (0.21)*** − 0.080 Exposure × Slope (0.55)***	0.81	0.124	0.04	28
(3) Log LZB = 2.388 − 0.155 Transparency (0.21)*** −0.0840 Exposure × Slope (0.61)***	0.80	0.127	−0.02	28
(4) Log LZB = 0.787 + 0.474 Plant biomass$^{0.5}$ (0.56)*** + 0.463 Log Chlorophyll (0.22)**	0.83	0.110	0.05	28
(5) Sediment water content % = 72.046 − 18.61 Log Slope (0.37)*** − 18.49 Log Exposure (0.19)**	0.65	11.29	0.09	28

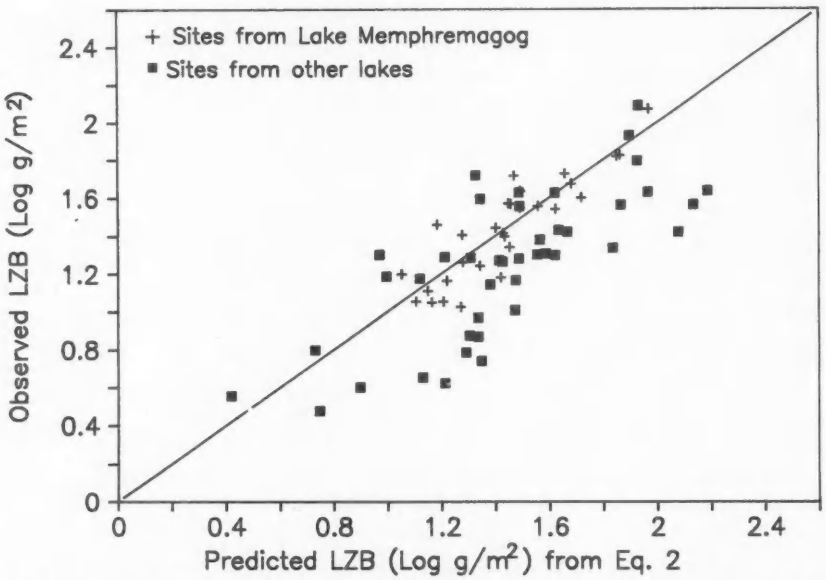

FIG. 4. Observed littoral zoobenthic biomass from Lake Memphremagog and other lakes (Table 1) as predictors from Equation 2 (Table 6).

colour, nor Secchi transparency was significantly correlated with the residuals. Conductivity was not correlated with the residuals; however, highly significant correlations were obtained for the concentration of calcium ($r = 0.49, p < 0.01$) and chloride ($r = -0.51, p < 0.01$), which were not significantly correlated with each other.

When all 71 sites were analyzed together, LZB was positively correlated with plant biomass, chlorophyll, and calcium concentration and negatively correlated with slope, transparency, and chloride concentration (Table 6). Multiple regression models obtained from this data set (Table 5) explained 79–83% of the variance in Log LZB. When plant biomass was included as a predictor, Equation 8 was obtained, and it contained significant contributions from plant biomass (+), slope (−), slope × exposure (−), chlorophyll (+), calcium (+), and chloride (−). When plant biomass was not entered, the best model

obtained was Equation 6 which explained 80% of the variance in LZB (Fig. 5) with significant contributions from slope (−), chlorophyll (+), calcium (+), chloride (−), and slope × exposure (−). The model that included plant biomass is very strongly influenced by collinearity ($d^2 = 0.41$ from collinear paths; Table 5), since slope is significantly correlated with plant biomass (Table 6). The contribution to these models from chloride was always more significant than its correlate sodium which was tested separately.

Sites located at stream mouths were compared with other littoral sites by ANCOVA. A categorical variable (1 for sites situated within stream delta versus 0 otherwise) was tested along with the predictors included in Equations 3 and 4 (within Lake Memphremagog analysis) and Equations 6 and 8 (the multilake analysis). Neither the regression coefficient for this categorical variable nor its interaction with predicted zoobenthic biomass was statistically significant for any of the models tested on the within Lake Memphremagog data set or the multilake data set. The relationship to the watershed area variable was also tested quantitatively by calculating the area draining to each sampling site (assumed to represent a 100-m segment of shoreline). This quantitative watershed variable (range 0.01–435 km²) was not significantly related to LZB in any of the analyses. Thus, coarse inputs from the watershed deposited in the littoral zone do not have a detectable effect on LZB, over and above that which might be operating through other factors already present in the models, such as, for example, local increases in phytoplankton or macrophyte biomass and/or changes in slope due to sediment deposition.

Since 29 (of 71) stations in the multilake analysis came from Lake Memphremagog, I tested whether these sites exerted special influence on the regression models obtained (Table 5). A dummy variable (value of 1 for Lake Memphremagog, 0 otherwise) was tested in all of the analyses and neither its regression coefficient nor its interaction with predicted LZB was ever significant. Thus the residuals from Lake Memphremagog sampling sites were no different from other sites once the chemical differences between the lakes had been accounted for.

Since 14 (of 71) stations in the multilake analysis came from outside of the Quebec Eastern Townships study area, and were sampled by different methods than those I used in the Quebec lakes, I tested the residuals from these sites for abnormal magnitude or bias. These residuals were, however, not significantly different in their absolute values, or more biased, than those from the Quebec sites for any of the models in Table 5. Since the 14 sites in question ranged over a fairly broad latitudinal range, I tested the residuals for a possible correlation with latitude and with mean annual air temperature (see Rasmussen and Kalff (1987) for data sources), but failed to detect any significant trends. Data on actual water temperatures were not available for most of these lakes.

For the 12 stations where sampling was done at three depths (d, the depth of maximum canopy, $0.5d$, and $1.5d$) covering most of the range of macrophyte growth on the littoral transect, the within-transect variance in Log LZB was only a small fraction of the between-transect variance ($F = 34.1$, $p < 0.001$). Furthermore, the estimate of zoobenthic biomass for the depth of maximum canopy height (Log LZB_d) was strongly related to the mean value (Log LZB) obtained by averaging over the three depths sampled on the littoral transect:

$$\overline{\text{Log LZB}} = 0.0286 + 0.9562 \text{ Log } LZB_d$$
$$R^2 = 0.95, \text{ SE of estimate} = 0.045, N = 12.$$

TABLE 5. Regression models for littoral zoobenthic biomass (LZB) in lakes listed in Table 1. Significance: $*p < 0.05$, $**p < 0.005$, $***p < 0.001$. The exposure × slope interaction was transformed as $(\text{exposure})^{0.25} \times (\text{slope})^{0.5}$. Molluscan biomass excludes shell weight and excludes unionid clams. All variables found not significant (n.s.) were dropped and the models retested without them. Both colour and Log Exposure were tested in all of these models and found not significant.

Predictors	Log LZB Eq. 6	LZB 0.1 Eq. 7	Log LZB Eq. 8	Log nonmolluscan biomass Eq. 9	Log molluscan biomass Eq. 10	Log sediment LZB Eq. 11	Log sediment LZB Eq. 12
Intercept	1.11***	1.29***	0.92***	0.95***	0.368	1.28***	1.15***
Chlorophyll *a* (Log μg/L)	+0.352 (0.14)***	+0.104 (0.14)***	+0.375 (0.13)***	+0.409 (0.18)***	n.s.	+0.446 (0.31)***	+0.431 (0.30)***
Slope (Log %)	−0.162 (0.34)***	−0.054 (0.35)***	−0.104 (0.03)*	−0.100 (0.30)***	−0.462 (0.14)***	−0.096 (0.13)**	n.s.
Exposure × Slope	−0.052 (0.04)***	−0.0160 (0.35)***	−0.033 (0.05)*	−0.052 (0.30)***	n.s.	−0.030 (0.13)**	−0.029 (0.09)**
Calcium (Log mg/L)	+0.431 (0.12)***	+0.134 (0.12)***	+0.341 (0.04)**	+0.330 (0.06)***	+0.905 (0.18)***	n.s.	n.s.
Chloride (Log mg/L)	−0.285 (0.10)***	−0.086 (0.09)***	−0.253 (0.08)***	−0.177 (0.08)**	−0.778 (0.20)***	−0.287 (0.14)***	−0.230 (0.11)***
Plant biomass $(kg/m^2)^{0.5}$	—	—	0.228 (0.09)***	—	—	n.s.	n.s.
Plant biomass/ height (kg/m^3)	n.s.	n.s.	n.s.	n.s.	n.s.	−0.252 (0.12)***	—
R^2	0.80	0.79	0.83	0.76	0.65	0.64	0.50
SE of estimate	0.163	0.051	0.160	0.168	0.356	0.158	0.182
$R^2 - \Sigma d^2$	0.10	0.09	0.41	0.14	0.03	−0.06	0.00
N	71	71	61	72	72	60	60

TABLE 6. Statistically significant ($p < 0.05$) correlation coefficients between variables in the complete data set (Table 1), $n = 71$. *$p < 0.01$.

Variable	Slope (Log %)	Exposure (Log km²)	Plant biomass (kg/m²)$^{0.5}$	Chlorophyll *a* (Log mg/m³)	Secchi disk (m)	Colour (mg Pt/L)	Calcium (Log mg/L)	Chloride (Log mg/L)	Sodium (Log mg/L)
LZB (Log g/m²)	−0.651*	n.s.	0.762*	0.674*	−0.558*	n.s.	0.512	n.s.	n.s.
Slope	—	n.s.	−0.591*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Exposure		—	−0.262	n.s.	n.s.	n.s.	0.539*	n.s.	n.s.
Plant biomass			—	0.486 (17)	n.s. (17)	n.s. (15)	n.s. (15)	n.s. (15)	n.s. (15)
Chlorophyll *a*				—	−0.671*	n.s.	0.576*	n.s.	n.s.
Secchi disk					—	−0.410	n.s.	n.s.	n.s.
Colour						—	n.s.	n.s.	n.s.
Calcium							—	n.s.	n.s.
Chloride								—	0.780*
df[a]	71	71	59	26	25	24	22	22	22

[a]Degrees of freedom are given in parentheses when they differ from the value at the bottom of the column.

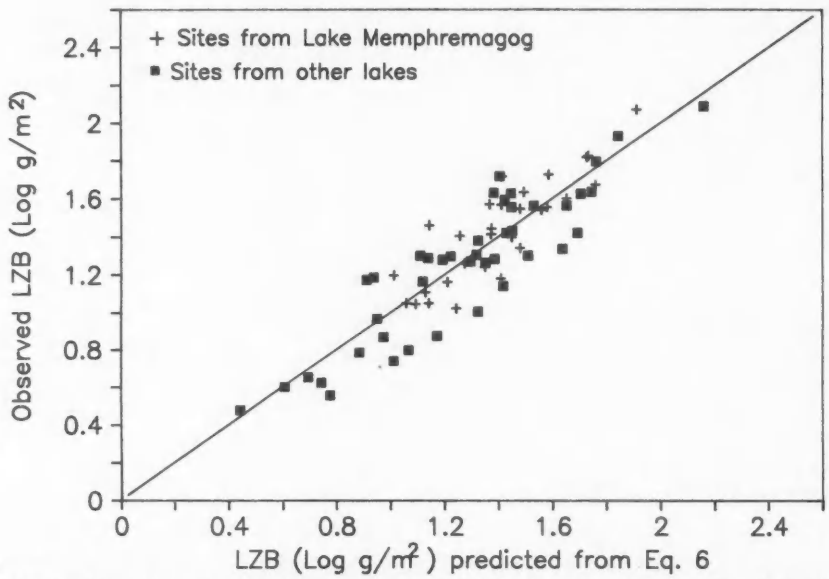

FIG. 5. Observed littoral zoobenthic biomass from Lake Memphremagog and other lakes (Table 1) versus predictions from Equation 6 (Table 5).

Thus, LZB_d can be used to represent mean littoral biomass on the transect, with only a slight correction. This analysis of within-transect variation in zoobenthic biomass is quite preliminary and does not take into account the nearshore zone where the substrate ranges from cobble to ooze supporting emergent macrophytes.

Discussion

Site-specific morphometric measurements contributed strongly to the prediction of LZB in this study, for both the Lake Memphremagog and multilake analyses. The strong decrease in zoobenthic biomass with increasing slope is at least partially a result of the reduced ability of steep slopes to retain fine, food-rich sediment. The relationship between slope and water content (Equation 5; also Håkanson 1977; Duarte and Kalff 1986) supports this interpretation. Slope was, however, a better predictor than either the sediment water content or the sediment organic matter content, both in this study and in that of Duarte and Kalff (1986). This suggests that other sediment characteristics (and perhaps a complex aggregate of factors) may be involved in the relationship between slope and littoral biota. Part of the relationship of slope to benthos is no doubt an indirect effect of the increased abundance of macrophytes in low-slope littoral environments (Duarte and Kalff 1986), since macrophytes contribute to substrate and food for zoobenthos (Rasmussen 1982; Davies 1982) and the reduced turbulence within macrophyte stands also enhances sedimentation and reduces resuspension rates (Prentki et al. 1979). There are, however, effects of slope over and above those operating through the abundance of macrophytes. Table 5 shows that entering plant biomass as a predictor reduces, but does not remove, the effect of the physical factors on zoobenthic biomass. Furthermore, Rasmussen and Kalff (1987) found highly significant regression coefficients for slope in relation to both sublittoral and profundal zoobenthos as well as LZB. Effects of slope on sediment properties, and therefore on the environment for benthic organisms, could also involve downgradient sliding or slumping of sediment, which has in some situations

been demonstrated (Prior and Suhayda 1979). Wind fetch is known to be a major determinant of wave height and the energy regime at the sediment–water interface (Smith and Sinclair 1972; Smith 1979; Hodgins et al. 1985). Since wave energy should be attenuated most abruptly on steep-sloped shores, it is not surprising that the slope × exposure interaction term makes a significant contribution to models predicting LZB.

Physical and trophic variables alone could not explain nearly as large a fraction of the variation in LZB in the multilake data set, as they did for the Lake Memphremagog sites. The two chemical variables, calcium and chloride concentration, explained most of the discrepancy, and the precision of the models derived from the multilake data set was only a slightly lower than for those derived from Lake Memphremagog data alone.

Interest in the relationship of the calcium concentration to aquatic productivity is not new. Pearsall (1921), Wilson (1939), Macan (1950, 1961, 1975), and Gorham et al. (1974) have shown that the concentration of calcium in water (hardness or alkalinity are very strong correlates) is associated with many aspects of biotic productivity. While this relationship likely involves broad-scale correlation between calcium and limiting nutrients, such as nitrogen and phosphorus, there is solid evidence for a direct contribution of alkalinity to biomass, growth, and marl production of submerged macrophytes (Hutchinson 1975; Duarte 1987). Much, but not all, of the calcium effect observed in this study was removed when plant biomass was entered as a predictor. Low concentrations of calcium (or hardness) have been cited frequently as limiting the distribution of many molluscan and crustacean species (Macan 1950, 1961; Clarke and Berg 1959; Hunter 1964; Aho 1966; McKillop and Harrison 1972; Økland 1969a, 1969b; Sutcliffe and Carrick 1973), and mechanisms involving both food availability, salt balance, and calcium accumulation for shell or cuticle growth have been proposed. Green (1971) identified the calcium–alkalinity complex as a major niche dimension in a discriminant analysis of sphaeriids and unionids. When I broke the zoobenthic biomass estimates into a molluscan and a nonmolluscan component and analyzed them separately, the calcium effect was much stronger for the molluscan fraction (Table 5).

Eight of the lakes sampled in this study have concentrations of chloride that are higher than the levels that would be expected on the basis of their total concentration of dissolved solids (as calculated from Hutchinson (1957) and Rodhe (1949)). Four of these lakes (Orford, Waterloo, D'Argent, and Brome) are surrounded by paved highways and receive substantial loads of deicing salt (mainly sodium chloride) each winter. Increases in sodium and chloride levels in lakes resulting from the use of deicing salt have been noted several times (Harmon and Sohacki 1978; Likens 1985). Septic tanks and other domestic sewage effluents (Provasoli 1969) are also common anthropogenic sources of sodium chloride that are probably contributing to increases in salt concentrations in Quebec lakes. Is the negative effect of chloride on LZB the same in lakes that are artificially chloride-rich and those that are naturally chloride-rich due to sea-spray or salt-rich geological deposits (e.g. Seneca and Cayuga Lakes, New York; Shaffner and Ogelsby 1978)? There is insufficient data to answer this in the present analysis, but it is an interesting question for future study.

The negative effects of chloride revealed by these regression analyses are not affected by the inclusion of plant biomass as a predictor, and they are much stronger for the molluscan component of the fauna than the nonmolluscan component (Table 5). Cvancara (1967) indicated that chloride had strong adverse effects on the abundance of many pelecypods in lakes of Minnesota and North Dakota, and Green (1971) found that sodium chloride (relative to other noncalcium salts) constituted a niche dimension upon which freshwater pelecypods segregate and that few species were abundant under elevated sodium chloride concentrations. Due to the prevalence of the use of sodium chloride for deicing roads in all temperate-latitude countries, the possibility of strong adverse effects of the salt on littoral invertebrates should not be ignored. Another possibility that deserves consideration is that other types of highway pollution such as heavy metals (Smith and Kaster 1983; Van Hassell et al. 1980) are contributing to adverse effects on littoral fauna and are correlated with chloride concentrations.

In a previous analysis of basin-average data obtained from the literature (Rasmussen and Kalff 1987; Equation 12) (RK) a model predicting LZB (Table 7) was obtained. The RK model is similar to models (cf. Equation 7) from the present study in that chlorophyll, slope, and exposure were significant predictors. However, there are also a number of notable differences. This study revealed significant effects of the two chemical predictors, calcium and chloride. Neither of these was tested in the previous analysis (RK), but I subsequently tested their relationship to the residuals from the RK model and found no significant relationship for either calcium or chloride. Water colour had a negative relationship to LZB in the RK model, and no such effects were found in this study, but this is likely a result

TABLE 7. Comparison of LZB estimates from this study to predictions from Equation 12 (Rasmussen and Kalff 1987). $LZB^{0.1} = 1.38527 + 0.10392$ Chlorophyll $(\mu g/L)^{0.5} - 0.04985$ Colour (mg Pt/L + 25)$^{0.5} - 0.01001$ Area (Ln km^2) $- 0.01850$ Slope (Ln%), $R^2 = 0.48$, SE of estimate (SD of residuals) $= 0.106$, $N = 84$.

	Based on basin-average LZB and slope estiamtes		Based on site-specific LZB and slope estimates	
	Total $LZB^{0.1}$ – predicted (Eq. 12)	Bottom $LZB^{0.1}$ – predicted (Eq. 12)	Total $LZB^{0.1}$ – predicted (Eq. 12)	Bottom $LZB^{0.1}$ – predicted (Eq. 12)
Mean departure from predicted value	+0.0863	+0.010	+0.074	+0.010
± SE	0.021	0.027	0.012	0.015
Significance of bias (t, p, N)	4.10, $p < 0.001$, 28	0.37, n.s., 20	6.23, $p < 0.001$, 71	0.66, n.s., 61
SD of departures	0.113	0.120	0.100	0.117
r between predicted and observed values	0.69 ($p < 0.001$)	0.62 ($p < 0.001$)	0.66 ($p < 0.001$)	0.56 ($p < 0.001$)

of the much lower variance in colour in the present data set. Finally, the models obtained in the present study are much more precise than the RK model; the residuals are about half as large, and R^2 values are about 30% higher. The use of site-specific LZB estimates and morphometric parameters in the present study, as compared with basin averages for the RK analysis, is the most likely reason that more covariation between LZB and morphometric factors was revealed in this study.

Predictions from the RK model were compared with LZB estimates from this study (Table 7), and most of the present estimates exceeded predictions from the RK model. This positive bias likely results from improved sampling of the epiphytic invertebrates in this study. Most of the LZB estimates available in the literature were obtained using remote-operated grabs of various kinds, which are sometimes ineffective on coarse substrates and do not quantitatively sample macrophytes. For comparison, I excluded the epiphytic component of my LZB estimates leaving only the bottom zoobenthic biomass, and these estimates were not significantly biased with respect to the RK predictions (Table 7). The regression models obtained in this study for sediment LZB alone (Equations 11 and 12) had lower R^2 and larger residuals than those obtained for total LZB (Equations 6 and 8), and they also showed no effect of calcium concentration. Thus the sediment LZB models from this study were more similar to the RK model than were the total LZB models, and this is reasonable considering the type of samples from which the RK model was derived.

In conclusion, this study has produced empirical models that relate littoral zoobenthic biomass to physical, chemical, and trophic predictors. The physical factors (slope and exposure) were generally most important (especially in explaining within-lake variation in LZB), but much of their effect was likely operating through effects on submerged macrophyte biomass. The proportion of variance in LZB explained in this study was much greater, and the residual variation was about half of that reported from a previous attempt to model LZB. This is probably a result of improving sampling, especially for epiphytic invertebrates, and the use of site-specific estimates of LZB, slope, and exposure, rather than basin averages which will reveal less covariation between these variables.

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Detection of Environmental Influence on Recruitment Using Abundance Data

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Environmental influences on recruitment are usually detected by correlation analysis using environmental and recruitment data. When recruitment data are unavailable, abundance data are sometimes used instead. An abundance time series can be described as a convolution of the recruitment time series, and consequently, when it is used as a proxy for recruitment, the magnitude of correlations is lower and the probability of both type I and type II errors is greater. Two approaches can restore the correlation between the environmental series and the recruitment series: (1) convolution of the environmental series and (2) deconvolution of the abundance series. Convolution of the environmental series increases the variance of computed correlation coefficients and can further increase the probability of type II errors. Deconvolution of the abundance series can recover the correlation between the environment and recruitment without increasing the variance of sample correlation coefficients or the probability of type II errors, as long as the deconvolution is stable and errors in the data are relatively small. When errors in the data are sufficiently large or the deconvolution is sufficiently unstable, the best results may be obtained by simply using abundance as a recruitment index. We evaluate application of the deconvolution technique to chinook salmon (*Oncorhynchus tshawytscha*) catch and spawner abundance series using both real and simulated data.

On détecte généralement les effets de l'environnement sur le recrutement par une analyse de corrélation faisant appel à des données sur le milieu et le recrutement. Lorsqu'on ne dispose d'aucune donnée sur le recrutement, on utilise parfois des données sur l'abondance. On peut décrire une série temporelle sur l'abondance comme une convolution de la série temporelle sur le recrutement et donc, lorsqu'on l'utilise à la place du recrutement, l'ampleur des corrélations est plus faible et la probabilité d'erreurs de type I et II est plus grande. Deux méthodes peuvent rétablir la corrélation entre la série sur l'environnement et la série sur le recrutement : 1) convolution de la série sur l'environnement, et 2) déconvolution de la série sur l'abondance. La convolution de la série sur l'environnement augmente la variance des coefficients de corrélation calculés et peut donc augmenter la probabilité des erreurs de type II. La déconvolution de la série sur l'abondance peut rétablir la corrélation entre l'environnement et le recrutement sans augmenter la variance des coefficients de corrélation des échantillons ou la probabilité des erreurs de type II, pour autant que la déconvolution est stable et que les erreurs dans les données sont relativement faibles. Lorsque les erreurs dans les données sont assez importantes ou que la déconvolution est assez instable, on peut obtenir les meilleurs résultats ne se servant simplement de l'abondance comme indice du recrutement. Nous évaluons l'application de la méthode de déconvolution aux séries sur les prises et l'abondance des géniteurs chez le saumon quinnat (*Oncorhynchus tshawytscha*) en utilisant à la fois des données réelles et des données de simulation.

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Fishery researchers and managers are frequently concerned with the impact of the environment on recruitment to managed populations. This concern may stem from (1) a desire to predict future abundance so that management of the population can be optimized, (2) a desire to determine the aspects of the environment affecting recruitment so that the environment itself can be managed to optimize recruitment (e.g. setting minimum instream flows or the timing of reservoir draw-down), or (3) a desire to gain a better understanding of the relative effect of population density and environment on recruitment. This relationship is frequently investigated by examining the correlations between environmental variables and estimated recruitment (Ricker 1975, p. 276), but in many cases, recruitment estimates are unavailable and some index of population abundance (e.g. catch or spawning escapement) must be used instead.

Suppose that we wish to test whether a recruitment series (x_t) is influenced by an environmental variable (u_t) using correlation analysis, but we lack observed recruitment data. Instead, we have an observed abundance index (y_t) that is the sum of contributions from several age-classes. When the age composition of abundance or catch is known for each year, recruitment can

be reconstructed by cohort analysis. However, we show here that even when the actual age composition of the abundance index for each year is not known, if the fraction of each cohort's recruitment that appears in the abundance record at each age is known (or can be estimated), recruitment can be estimated. Each age-class in the abundance index can be written as a certain fraction (β_i for the ith age-class) of past recruitment (x_{t-i}). Current abundance can then be written as a weighted sum, or *convolution*, of past recruitments. The technique described here, *deconvolution*, essentially inverts the convolution to obtain values of x_t from y_t.

Computing correlations using abundance data instead of recruitment data can change both the magnitude of computed correlation coefficients and the computed significance levels. We explain this effect here and examine two approaches for recovering correlations between the environment and recruitment from environmental and abundance time series. We show that one approach, convolution of the environment, can increase the magnitude of the correlation, but that it also widens the confidence limits of estimated correlation coefficients. Under suitable conditions, a second approach, deconvolution of abundance, can increase the correlation without affecting the confidence limits of estimated correlation coefficients. We then review past use of convolved time series in fisheries and demonstrate the use of deconvolution using catch and spawning escapement data for central California chinook salmon (*Oncorhynchus tshawytscha*). We evaluate the performance of this application of deconvolution using Monte Carlo simulations.

Throughout the development and analysis of the methodology presented here, we will assume that both the environmental time series and the recruitment time series are not autocorrelated and that recruitment is correlated with the environment at only one lag. We make these assumptions to simplify the analysis, but similar conclusions can be derived if they do not hold.

Effects of Autocorrelation in Time Series

To demonstrate how the use of abundance data decreases the correlation between environmental series and recruitment series, we assume that we have an environmental time series, u_t ($t=1,2,\ldots,n$) with mean μ_u and variance σ_u^2, and that this environmental variable determines recruitment in year $t+\tau$, $x_{t-\tau}$, with an independent random process error ϵ_t. Thus

$$(1)\quad x_{t+\tau} = (c + \epsilon_t)\, u_t$$

where c is a constant and the values of ϵ_t are normally distributed with mean 0.0 and variance σ_ϵ^2. We define recruitment as cohort abundance in the year when a cohort first contributes to the abundance index. The coefficient of linear correlation between u and x, at any lag i, is then given by

$$\rho_{ux}(i) = \begin{cases} \dfrac{c\sigma_u}{\sigma_x}; & i=\tau \\ 0; & \text{elsewhere} \end{cases}$$

where

$$\sigma_x^2 = c^2\,\sigma_u^2 + \sigma_\epsilon^2(\sigma_u^2 + \mu_u^2).$$

The assumption of a bivariate normal distribution for linear correlation is violated by the form of the errors in equation (1), but a multiplicative error term is used here because it is more typical of fisheries data than additive errors (cf. Peterman 1981).

Suppose that we also have a time series of abundance data, y_t, that includes several age-classes. The abundance index can be catch from a fishery with knife-edge selectivity at a certain age or at a certain size. For the age-specific case,

$$(2a)\quad y_t = h \sum_{i=a}^{A} n_{i,t}$$

where $n_{i,t}$ is the number at age i and time t, h is the harvest fraction, a is the age of recruitment, and A is the maximum age. For constant annual natural survival, s, this becomes

$$(2b)\quad y_t = h \sum_{i=0}^{m} x_{t-i} s^i (1 - h)^i$$

where $m = A - a$. For the case in which selection is based on size and cohorts have a size distribution of non-zero width,

$$(2c)\quad y_t = h \sum_{i=0}^{m} p_{i+a} x_{t-i} s^i \sum_{j=0}^{i} p_{j+a} (1 - h)^{i-j}$$

where p_i, vulnerability, is the fraction of a cohort entering the sampling range at age i (cf. Botsford 1986a, 1986b).

For each of these cases, as long as the mortality rates (h and s) and vulnerabilities (p_i) do not vary with time, the abundance index is a weighted moving sum of past recruitment or a convolution of the recruitment series and the weighting function:

$$(3)\quad y_t = \sum_{i=0}^{m} \beta_i x_{t-i}$$

where

$$\beta_i = \begin{cases} hs^i(1-h)^i & \text{for the age-specific case} \\ hp_{i+a}s^i \sum_{j=0}^{i} p_{j+a}(1-h)^{i-j} & \text{for the size-specific case.} \end{cases}$$

Similar weighting functions can be derived for other abundance indices (e.g. abundance estimates from survey cruises, estimates of spawning biomass, etc.), and similar, although more complicated, weighting functions are possible for nonstationary convolutions (i.e. time-varying mortality and harvest rates). As a result of the convolution of recruitment with the weighting function, abundance is a smoothed version of recruitment with autocorrelation added by the removal of much of the high-frequency variability (Botsford 1986a, 1986b; Welch 1986).

We are interested in the correlation between the environmental series and the recruitment series, $\rho_{ux}(\tau)$, but since we do not know recruitment, the usual procedure is to estimate the correlation between the environmental series and abundance, $\rho_{uy}(t)$, from our data series of abundance and the environment. This results in a distorted version of $\rho_{ux}(\tau)$. By substitution of equation (3) into the definition of the coefficient of linear correlation, we obtain

$$(4)\quad \rho_{uy}(t) = \frac{\sum_{j=0}^{m} \beta_j \rho_{ux}(t-j)}{\sqrt{\sum_{j=0}^{m} \sum_{k=0}^{m} \beta_j \beta_k \rho_{xx}(j-k)}}$$

which has a maximum at lag $\tau + i^*$, where i^* is the index of the largest β_i. The magnitude of $\rho_{uy}(\tau + i^*)$ is less than that of $\rho_{ux}(\tau)$ by the factor $\beta_{i^*} / \sqrt{\sum_{j=0}^{m} \beta_j^2}$ and we increase the probability of not detecting relationships that are real (type I errors) because they are obscured by the convolution of recruitment reflected in the abundance time series (Fig. 1a and 1b). In addition, we increase the risk of falsely detecting correlations at lags other than $\tau + i^*$ (type II errors) because $\rho_{xx}(\tau)$ appears in the numerator of equation (4) at lags other than $\tau + i^*$.

There are two approaches to estimate $\rho_{ux}(\tau)$ from the time series of environment and abundance when the weights of the convolution, β_i, are known. The first approach is to apply the same convolution to the environment and the second is to use

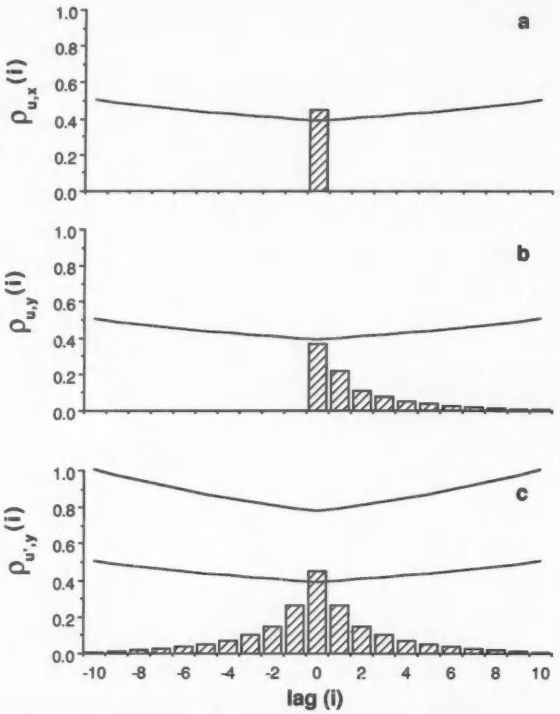

FIG. 1. Crosscorrelations (hatched bars) between environmental and recruitment series and upper 95% confidence limits for sample correlation coefficients (solid lines) based on a sample of 25 yr in length. (a) Crosscorrelation between the environment and recruitment with $\tau = 0$ and $\rho_{uu}(\tau) = 0.5$. (b) Crosscorrelations between the environment and abundance when the convolution is a geometric series (i.e. equation (2b)). (c) Crosscorrelations between convolved environment and abundance; the lower line is the upper 95% confidence limit from Fig. 1a and 1b and the upper line is the upper 95% confidence limit when intraseries correlations are taken into account.

the inverse convolution to recover the recruitment series from abundance. Both of these approaches have limitations and drawbacks that will be examined in detail.

Convolution of the Environment

The correlation between the environment and recruitment can be restored by convolving the environmental series to obtain

$$(5)\quad u'_t = \sum_{i=0}^{m} \beta_i u_{t-i}.$$

By substitution of equations (3) and (5) into the definition of the coefficient of linear correlation, we obtain

$$(6)\quad \rho_{u'y}(t) = \frac{\rho_{uu}(\tau) \sum_{j=-\infty}^{\infty} \beta_j \beta_{j-t+\tau}}{\sum_{j=0}^{m} \beta_j^2}.$$

This restores the correlation between environment and recruitment at lag τ (i.e. $\rho_{u'y}(\tau) = \rho_{ux}(\tau)$ but has the disadvantage of introducing autocorrelations at lags other than zero and increasing the crosscorrelations at lags other than τ. The former increase the variance of estimated sample correlation coefficients, $r_{u'y}$t, and the latter increase the probability of type II errors at inappropriate lags.

The variance of sample correlation coefficients can be approximated by (L. W. Botsford and T. Wainwright, unpubl.)

$$(7)\quad \mathrm{Var}[r_{xy}(t)] \approx \frac{1}{n} \sum_{i=1-n}^{n-1} \left(\frac{|i|}{n}\right) \times [\rho_{xx}(i)\rho_{yy}(i) + \rho_{xy}(t+i)\rho_{xy}(t-i)] - \frac{2r_{xy}(t)}{n} \sum_{i=1-n}^{N-1} \left(1 - \frac{g}{n}\right) \times [\rho_{xy}(i)\rho_{yy}(i-t) + \rho_{xx}(i)\rho_{xy}(t-i)] + \frac{r_{xy}^2(t)}{2n} \sum_{i=1-N}^{N-1} \left(1 - \frac{|i|}{N}\right)[\rho_{xx}^2(i) + 2\rho_{xy}^2(i) + \rho_{yy}^2(i)]$$

where

N = length of the data series
n = number of data pairs at lag t (i.e. $N - |t|$)

$$g = \begin{cases} -i : i<0 \\ 0 : 0 \leq i \leq |t| \\ i-|t| : i>|t|. \end{cases}$$

(This expression is similar to that of Bartlett (1966) except that, since it does not assume a large sample size, it includes a triangular weighting function in each sum and finite limits of summation (Botsford 1987). It is the corrected version of expression (1) in Johnson et al. (1986).) Because the null hypothesis that we are testing is that $\rho_{xy}(t) = 0.0$, and we have assumed that all crosscorrelations between convolved series result from a single crosscorrelation between the environment and recruitment, we can simplify expression (7) as

$$(8)\quad \mathrm{Var}[r_{xy}(t)] \approx \frac{1}{n} \sum_{i=1-n}^{n-1} \left(1 - \frac{|i|}{n}\right) \rho_{xx}(i)\, \rho_{yy}(i).$$

It is apparent from expression (8) that the variance of sample crosscorrelation coefficients between any two series, when at least one series has an autocorrelation function that is zero at all non-zero lags, is approximately $1/n$. Crosscorrelations between the environment and recruitment or the environment and abundance both involve the environmental series which we have assumed to have an autocorrelation function that is zero at all non-zero lags. However, when we calculate correlation coefficients for the convolved environmental series and abundance, both series have non-zero autocorrelations at non-zero lags and the variance of the sample correlation coefficients increases by

$$(9)\quad \mathrm{Var}[r_{u'y}(\tau)] \approx \mathrm{Var}[r_{uy}(\tau + i^*)] \sum_{i=m}^{m} \left(1 - \frac{|i|}{n}\right) \left(\frac{\sum_{j=0}^{m} \beta_j \beta_{j+i}}{\sum_{j=0}^{m} \beta_j^2}\right)^2.$$

From equation (4) using the fact that $\rho_{u'y}(\tau) = \rho_{uu}(\tau)$, it is apparent that

$$(10)\quad \rho_{u'y}(\tau) = \rho_{uy}(\tau + i^*) \frac{\sqrt{\sum_{i=0}^{m} \beta_i^2}}{\beta_{i^*}}.$$

Although it is not readily apparent from expressions (9) and (10), the increase in the standard deviation of sample correlation coefficients is typically more than the increase in the magnitude of the correlation (Fig. 1c). Consequently, even though convolution of the environmental series restores the correlation in which we are interested, if we account for the autocorrelations

introduced by this approach, we increase the probability of type I errors. If we ignore the autocorrelations introduced by convolution of the environment, then we increase the probability of type II errors at inappropriate lags.

If the autocorrelation function is not white as assumed (i.e. if the autocorrelation function of the environmental series is non-zero at non-zero lags), analysis of the effects of convolving the environment is still possible, although more difficult. The effects in any individual case will depend on the autocorrelation function of the particular environmental series used, but the degree of autocorrelation in the convolved environmental series will typically be greater than the degree of autocorrelation in the raw environmental series. Consequently, the results will usually be similar to the uncorrelated case presented above, and it is unlikely that convolution of the environment will decrease the probability of either type I or type II errors.

Deconvolution of Abundance

The correlation between the environment and recruitment can also be restored by deconvolving abundance to remove the effects of summing over age-classes. There is an extensive literature dealing with convolution and deconvolution in the fields of statistics (Box and Jenkins 1976), systems analysis (Freeman 1965; Lewis 1977), and signal processing (Blass and Halsey 1981; Kanasewich 1973; Rice 1962). Perhaps the most useful of this literature for the deconvolution problem is found in the field of geophysics and deals with the problem of interpreting seismic traces in fossil fuel exploration (Kanasewich 1973; Rice 1962).

Recruitment can be estimated from abundance only if the deconvolution is stable. If the recruitment estimates from deconvolution, $\hat{x}_t$, converge to the real values of recruitment, x_t, as t increases when there are no errors in the estimates of y_t or β_i, then the deconvolution is by definition stable, and we will refer to situations in which $\hat{x}_t$ converge to x_t rapidly as more stable than slower convergence. Thus even in the absence of estimation errors in β_i and y_t, the accuracy of recruitment estimates obtained by deconvolution of abundance data depends on how stable the deconvolution is and on how close the initial recruitment estimate, $\hat{x}_1$, is to the actual value of recruitment in the first year, x_1.

The stability of deconvolution can be evaluated by examining the z-transform of the corresponding convolution, which is obtained by writing the convolution as a polynomial in the time domain:

$$(11)\quad f(z) = \beta_0 + \beta_1 z + \beta_2 z^2 + \ldots + \beta_m z^m$$

where z is the backward shift operator, $z^n a_t = a_{t-n}$ (cf. Box and Jenkins 1976). If all the roots of this polynomial lie outside the unit circle, then the convolution as represented by equation (11) is said to be minimum phase and has a stable inverse in positive powers of z. Therefore, the recruitment estimates obtained from a minimum phase convolution will depend only on past abundance. If all the roots of equation (11) lie inside the unit circle, the convolution is said to be maximum phase and a stable inverse can be obtained by multiplying equation (11) by z^{-m}. The stable inverse of a maximum phase convolution will involve only negative powers of z, and recruitment estimated by deconvolution will depend only on future values of abundance. If equation (11) has roots inside and outside the unit circle, then the convolution is mixed phase and a stable inverse can be obtained by multiplying equation (11) by z^i for some i such that $0 > i > -m$. The stable inverse of a mixed phase convolution will involve both positive and negative powers of z (Kanasewich 1973). For a mixed phase convolution, recruitment estimates will depend on both past and future values of abundance. If equation (11) has roots that lie on the unit circle, then no stable inverse exists (Rice 1962).

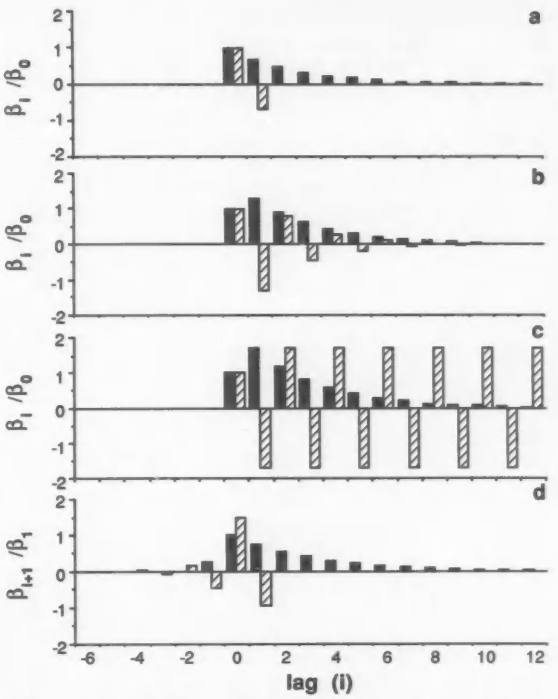

FIG. 2. Examples of deconvolutions (hatched bars) that correspond to catch or abundance convolutions (solid bars). (a) Coefficients of a geometric series and its inverse; this convolution would describe a fish population in which mortality rates are constant and all ages are equally available. (b) Convolution as in Fig. 2a with the youngest age-class less available than subsequent age-classes. (c) Convolution as in Fig. 2b where youngest age-class is half as available as older age classes and deconvolution is unstable. (d) Convolution as in Fig. 2c where youngest age-class is less than half as available as older age-classes and the convolution has been time-shifted to obtain a stable inverse.

These stability conditions can best be illustrated by a few simple hypothetical examples. In a population where natural and fishing mortality rates are constant and all age-classes present in the catch are equally vulnerable to the fishery (i.e. cohorts are either fully vulnerable to the fishery or not vulnerable at all), the coefficients in $f(z)$ constitute a geometric series. The deconvolution for the catch series from such a population consists of only two terms (Fig. 2a). If the youngest age-class is not fully available to the fishery, higher order terms arise in the deconvolution and become increasingly important as availability of the youngest age-class decreases (Fig. 2b). When the availability of the youngest age-class is half that of subsequent age-classes, $f(z)$ has a root on the unit circle, and the deconvolution is unstable. The coefficients of higher order terms in this unstable deconvolution are constant in magnitude (Fig. 2c).

If the availability of the first age-class is less than half that of older age-classes (so that no roots fall on the unit circle, but one root is now within the unit circle), then the coefficients of the convolution must be shifted so that the second age-class is at lag zero to obtain a stable inverse (Fig. 2d).

Convolution may be mathematically represented in a variety of forms. Thus far we have represented it as a weighted moving average process. In considering deconvolution, it is conceptually and computationally simpler to represent convolution in matrix form. Equation (3) can be rewritten as

$$\bar{\boldsymbol{X}} = \bar{\boldsymbol{B}}\bar{\boldsymbol{X}} \tag{12}$$

where

$$\bar{\boldsymbol{X}} = \begin{bmatrix} x_{-m+1} \\ x_{-m+2} \\ \vdots \\ x_0 \\ x_1 \\ x_2 \\ \vdots \\ x_n \end{bmatrix}, \qquad \boldsymbol{Y} = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}$$

and

$$\bar{\boldsymbol{B}} = \begin{bmatrix} \beta_m & \cdots & \beta_1 & \beta_0 & 0 & 0 & \cdots & 0 \\ 0 & \beta_m & \cdots & \beta_1 & \beta_0 & 0 & \cdots & 0 \\ \vdots & \ddots & & \ddots & \ddots & \ddots & & \vdots \\ & & \beta_m & & \ddots & & \beta_1 & \beta_0 \end{bmatrix}$$

After removing columns from the left side of $\bar{\boldsymbol{B}}$ to obtain a square matrix and similarly truncating $\bar{\boldsymbol{X}}$ to the same order as $\boldsymbol{Y}$ and the truncated $\bar{\boldsymbol{B}}$, we have

$$\boldsymbol{B} = \begin{bmatrix} \beta_0 & 0 & 0 & \cdots & 0 \\ \beta_1 & \beta_0 & 0 & & \vdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ \beta_m & & \ddots & \ddots & 0 \\ 0 & \ddots & & \ddots & \vdots \\ \vdots & \ddots & \ddots & & 0 \\ 0 & \cdots & 0 & \beta_m & \beta_0 \end{bmatrix} \quad \text{and} \quad \boldsymbol{X} = \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{bmatrix}$$

$\boldsymbol{B}$ is an invertible square matrix and the abundance series can now be deconvolved by

$$\hat{\boldsymbol{X}} = \boldsymbol{B}^{-1}\boldsymbol{Y} \tag{13}$$

where $\hat{\boldsymbol{X}}$ is the column vector of recruitment estimates obtained by deconvolution of abundance.

Truncation of the convolution matrix assumes that the recruitment series ($\bar{X}$) takes on the value of zero outside the bounds of $\boldsymbol{X}$. In a case with three age-classes, after truncation, we would have

$$\begin{aligned} y_1 &= \beta_0 x_1 \\ y_2 &= \beta_0 x_2 + \beta_1 x_1 \\ y_i &= \beta_0 x_i + \beta_1 x_{i-1} + \beta_2 x_{i-2} \quad \text{for } i = 3, 4, \ldots, n. \end{aligned}$$

Because the expressions for y_1 and y_2 should consist of three terms, they will produce biased values of $\hat{\boldsymbol{X}}$ on the end that has been truncated. To satisfy the expression for y_1, $\hat{x}_1$ will be overestimated, and the bias in $\hat{x}_1$ will influence subsequent recruitment estimates. The accuracy of these estimates can be improved by adjusting the elements of $\boldsymbol{B}$ in rows that have lost non-zero elements through truncation, to compensate for the lost terms. We adjusted the elements of $\boldsymbol{B}$ using

$$b_{ij} = \frac{\bar{b}_{ij} \sum_{j=m+1}^{n} \bar{b}_{ij}}{\sum_{j=1}^{n} \bar{b}_{ij}} \tag{14}$$

where b_{ij} denotes the element in the ith row and jth column of $\boldsymbol{B}$, and $\bar{b}_{ij}$ denotes the corresponding element in $\bar{\boldsymbol{B}}$. This, in effect, approximates the assumption of average recruitment outside the bounds of our truncated series.

Although knowledge of the stability conditions is useful for theoretical considerations, we need not solve for the roots of equation (11) to evaluate stability in practice. Stability can be determined empirically by inspection of $\boldsymbol{B}^{-1}$. When the deconvolution is stable, the elements of $\boldsymbol{B}^{-1}$ eventually decrease in magnitude with distance from the main diagonal. The faster the elements of $\boldsymbol{B}^{-1}$ approach zero, the faster $\hat{x}_t$ will approach x_t as t increases, and the greater the stability of the deconvolution.

The phase of the convolution can also be determined empirically by shifting the lag between the recruitment and abundance series until a stable deconvolution is obtained. In practice, these time shifts can be accomplished by removing columns from the right side of $\bar{\boldsymbol{B}}$ and the same number of elements from the bottom of $\bar{\boldsymbol{X}}$ prior to the truncation performed to make $\boldsymbol{B}$ a square matrix. Columns can be sequentially removed from the right side of $\bar{\boldsymbol{B}}$ until a stable inverse of $\boldsymbol{B}$ is obtained. The number of columns removed from the right side of $\bar{\boldsymbol{B}}$ to obtain a stable inverse corresponds to the number of roots of equation (11) that lie within the unit circle.

In addition to boundary conditions and the relative stability of the deconvolution, observational errors in the abundance data and errors in estimates of β_is (i.e. process errors) also affect the accuracy of recruitment estimates obtained by deconvolution. The effects of observational errors by themselves can be easily evaluated. If we assume that the coefficients in the convolution are known exactly, then

$$\mathrm{Var}(\epsilon_{\hat{x}}) = \mathrm{Var}(\epsilon_y) \sum_{i=-\infty}^{\infty} (\beta_i^{-1})^2 \tag{15}$$

where β_i^{-1} denote coefficients of the inverse of equation (11), not the inverses of the coefficients of equation (11), $\epsilon_{\hat{x}}$ are errors in the recruitment estimates, and ϵ_y are the errors in the abundance estimates. Because the variance of errors in recruitment estimates is proportional to the sum of the squared coefficients in the inverse convolution, the errors in recruitment estimates increase as the relative stability of the inverse convolution decreases. When the inverse convolution is unstable, it is still theoretically possible to obtain recruitment estimates if there are no errors in the data, but when errors are present in the abundance data (i.e. in any practical application), the variance of errors in the recruitment estimates is infinite.

Process errors are due to differences between the population processes that ultimately lead to the observed abundance index and the behavior described by the convolution. Because process errors can arise from a variety of sources and in a variety of forms, they are much more difficult to evaluate than observational errors. In addition, process errors affect the propagation of observational errors through deconvolution. The approach we have taken is to evaluate the effects of process and observational errors in nonstationary convolutions using Monte Carlo simulations.

Examples of Past Use of Convolved Time Series Data

Both convolution of environmental series and deconvolution of abundance series have been used by investigators in the past. When testing for an influence of fishing effort on catch, Van Hyning (1973) computed the correlation between a two-point moving average of effort and the run size of Columbia River chinook salmon, assuming that catch was composed primarily of age 3 and age 4 fish. Johnson et al. (1986) used two-point moving averages of environmental variables for some of their correlations between various environmental variables and abundance of the Dungeness crab (*Cancer magister*) at different locations on the Pacific coast. However, they accounted for the increased variability in estimated correlation coefficients by using expression (7) to estimate the significance of some correlation coefficients (their correlated series test). They also used $1/n$ instead of expression (7) to approximate the variance of sample correlation coefficients (their uncorrelated series test) and found that in their case the test of significance used did not affect the conclusions drawn.

Van Hyning (1973), Dettman et al. (1986), and Reisenbichler (1986) all attempted to deconvolve abundance indices, in the form of escapement estimates, for chinook salmon, using the same basic approach. They assumed that the age composition of spawning runs was constant (or piecewise constant) with time (i.e. as it would be if recruitment and mortality rates were constant). Using this assumption, they broke each spawning run into its component age-classes and added up the results for each cohort. In essence, this approach attempts to deconvolve the spawning escapement using $f(z^{-1})$ rather than $f^{-1}(z)$ and is equivalent to substituting the transpose of $\tilde{\boldsymbol{B}}$ for $\tilde{\boldsymbol{B}}^{-1}$ in equation (13). Instead of removing the effects of averaging, this incorporates more age-classes into the moving average and further increases the probability of both type I and type II errors. Both Van Hyning (1973) and Reisenbichler (1986) found some significant correlations between their recruitment estimates and environmental variables, but the perceived significance of these correlations may have been due to long-term trends in both series rather than year-to-year covariation between the data series.

When the convolution is a geometric series, abundance can be deconvolved by a weighted first differencing of the abundance series (i.e. Fig. 2a). This occurs when adult survival is constant and there is no partial recruitment. Partial recruitment can be eliminated by defining recruitment to be the number of fish that become fully available to the fishery rather than year-class strength. Using this definition, recruitment in each year may include several age-classes. This approach has been used to obtain recruitment estimates for northern anchovy (*Engraulis mordax*) (Huppert et al. 1980) and the Dungeness crab (McKelvey et al. 1980) and is the basis of the SHOT estimates used by ICES (1985). Walter and Hoagman (1975) took this approach one step further and obtained estimates of year-class strength by differencing the recruitment estimates. They applied their method to commercially fished species in Green Bay, Lake Michigan.

Walter and Hoagman (1975) described recruitment as a convolution of year-class strength and abundance as a convolution of recruitment in terms of difference equations. In a two-step process, they deconvolved catch to obtain recruitment and deconvolved recruitment to obtain year-class strength. However, it is not necessary to separate these component convolutions. Instead, the abundance index can be represented in a form similar to equation (3). In this case, the z-transform of the total convolution (equation (15)) can be obtained by simply multiplying the z-transforms of the two component convolutions, and year-class strength can be obtained by deconvolving the abundance index in one step.

Walter and Hoagman (1975) did not discuss errors in their estimates of year-class strength. In the one example of actual data that they presented, the recruitment convolution had a root on the unit circle and consequently had no stable inverse. They stated that even though the deconvolution was an infinite series with coefficients that were of constant magnitude, the correct sequence of year-class strengths could be obtained by simply choosing an appropriate constant as a boundary condition on recruitment. While this is correct when there are no errors present, it is readily apparent from equation (15) that if any errors are present in the abundance data, the variance of errors in estimates of year-class strength is infinite.

Central Valley Chinook Salmon as an Example of Deconvolution

Chinook salmon from California's Central Valley stock provide a good example of the use of deconvolution. The stock is reasonably well segregated from other stocks at sea and it is exploited by sport and commercial troll fisheries. Three data series are available for estimation of recruitment: commercial catch, sport catch, and spawning escapement. This allows comparison of recruitment estimates from three different abundance indices for the same stock. Catch and effort data are available for the past 25 yr in both fisheries, and age-specific fishing vulnerabilities and spawning probabilities estimated by separable virtual population analysis of marked hatchery fish (Kope 1987) allow the construction of nonstationary convolution matrices.

Methods

To construct the convolution matrices, commercial effort in annual number of deliveries and sport effort in number of angler days were used. Catch, effort, and spawning escapement data from 1971 through 1986 were taken from Pacific Fishery Management Council (PFMC) reports. Data for the period from 1962 through 1970 were obtained from L. B. Boydstun (California Department of Fish and Game, Anadromous Fisheries Branch, unpubl. data). Prior to 1971, few data were collected for the sport fishery, and data were reported as catch and angler success rates. We estimated sport effort from these data by dividing catch by angler success. Consequently, sport catch and effort data for the period from 1962 through 1970 are not as accurate as the commercial fishery data or the sport fishery data collected after 1971.

Three abundance data series were used: commercial catch south of Point Arena, sport catch south of Point Arena, and annual spawning escapements for all Central Valley runs combined. Point Arena is the boundary used by the PFMC to separate the Central Valley chinook stock from more northern stocks at sea, and the bulk of the catch south of Point Arena comes from the Central Valley stock (PFMC 1983). A small, but varying, portion of the Central Valley stock is landed north of Point Arena, and the catch south of Point Arena contains a varying portion of more northerly stocks. Two measures of spawning escapement were available: total spawners and adult spawners (i.e. age 3 and older) were used. Adults were separated from jacks (age 2 spawners) on the basis of length. The reliability of this separation is not known, since changes in the length-at-age distributions and changes in the proportion of jacks in the spawning escapement interact to affect the number of jacks classified as adults and the number of adults classified as jacks.

Convolution matrices were constructed by substituting the observed commercial and sport effort into a cohort model of chinook salmon and calculating the distribution of each year-class in subsequent catches and spawning escapements (details are given in the Appendix). The convolutions for both catch series, and for total spawning escapement, are mixed phase due to the poor representation of age 2 fish (Table 1). Since mixed phase deconvolutions are two-sided, they generally have a greater potential for amplifying observational errors than minimum phase deconvolutions. For this reason, minimum phase approximations for the convolutions in commercial and sport catch, obtained by omitting the coefficient for age 2 fish, were examined in addition to the complete mixed phase convolutions.

Because we have three abundance series that represent different convolutions of the same recruitment series, we can obtain three different estimates of the same recruitment series. We can also find a single recruitment series that, when convolved, provides the best fit to all three abundance series under some criteria for goodness of fit (e.g. least-squares, maximum likelihood). However, a natural way of combining the series is to simply add the abundance series together and add the three convolution matrices together to obtain a combined abundance index and a combined convolution matrix. A combined estimate for recruitment can then be obtained by equation (13). In terms of a criterion function, this corresponds to setting the average error over all three abundance series to zero in each year.

Error analysis was performed on all individual deconvolutions and on the combined recruitment estimates using Monte Carlo simulations (details are given in Appendix). Each simulation consisted of 1000 trials with artificial data sets. In each trial a normally distributed random recruitment series was generated and artificial abundance series of 25 data points were constructed using nonstationary convolutions (i.e. they involved time-varying harvest rates). The convolution matrices were generated in the same way that was used for the actual data (see Appendix) using life-history parameters from Kope (1987) and simulated commercial and sport effort data randomly generated from normal distributions with the same means and standard deviations observed in the fisheries. Observational errors were introduced by multiplying the artificial abundance data by normally distributed, independent random numbers with mean = 1.0 and standard deviation = 0.1 to obtain a 10% coefficient of variation of errors in the abundance series. Process errors were simulated by similarly multiplying the elements of the convolution matrix ($\tilde{B}$) for each artificial abundance series by normally distributed, independent random numbers to obtain a 10% coefficient of variation in the convolution coefficients. The artificial abundance series were then deconvolved using the same assumptions and methods as were used to deconvolve the real data. Correlations were calculated between recruitment and all indices in each trial, and the correlations reported for the Monte Carlo simulations are averages of the results from 1000 trials.

TABLE 1. Stationary approximations to convolutions for central California chinook salmon.

Data series	β_0	β_1	β_2	β_3
Commercial catch	0.00270	0.28600	0.05200	0.00120
Sport catch	0.03800	0.08000	0.00620	0.00016
Spawners	0.04500	0.16250	0.07860	0.00195

To evaluate and compare the value of various deconvolution approaches, the deconvolved recruitment estimates were compared with the raw abundance data and catch-per-unit-effort (CPUE) as indices of recruitment. Assuming that observational and process errors are independent of the environment, the correlation between each series and the recruitment series it was generated from indicates how well that series would perform in detecting correlations between the environment and recruitment.

Results and Discussion

The deconvolved recruitment estimates perform extremely well in the Monte Carlo simulations when there are no observational or process errors (Table 2). In all cases the deconvolved estimates are almost perfectly correlated with recruitment and perform much better than the raw abundance series. Convergence errors on the ends of the deconvolved series result in some correlations less than 1.0. The minimum phase approximation for commercial catch performs about the same as the mixed phase deconvolution, but in the sport catch the minimum phase approximation is noticeably worse than the mixed phase deconvolution.

When errors are included, the increase in accuracy gained by using deconvolutions instead of raw abundance data

TABLE 2. Correlations of simulated recruitment indices with recruitment.

	Source of errors			
Index	None	Observation	Process	Both
Commercial catch	0.71	0.68	0.68	0.65
Commercial CPUE	0.94	0.84	0.87	0.80
Deconvolution	1.00	0.89	0.92	0.83
Minimum phase approximation	1.00	0.89	0.92	0.83
Sport catch	0.53	0.50	0.50	0.49
Sport CPUE	0.88	0.77	0.81	0.72
Deconvolution	0.99	0.79	0.87	0.73
Minimum phase approximation	0.90	0.77	0.82	0.72
Total Spawners	0.72	0.63	0.67	0.60
Deconvolution	0.98	0.58	0.72	0.50
Adult Spawners	0.74	0.64	0.69	0.61
Deconvolution	0.99	0.75	0.87	0.69
Combined deconvolution	1.00	0.91	0.95	0.87

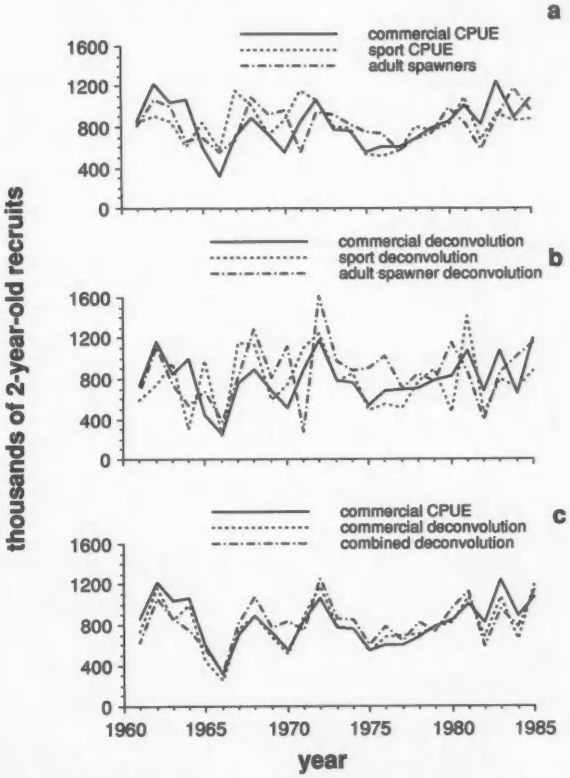

FIG. 3. (a) Recruitment estimates obtained by scaling commercial CPUE, sport CPUE, and adult spawning escapement. (b) Recruitment estimates obtained by deconvolution of commercial catch, sport catch, and adult spawning escapement. (c) Comparison of the three recruitment indices that performed best in the Monte Carlo simulations.

decreases. However, with the exception of total spawners, the deconvolutions consistently perform better than raw abundance as indices of recruitment. The poor performance of the deconvolution of total spawners is due to the relatively low stability of this deconvolution and the resultant amplification of observational errors. Also, the increase in stability of the minimum phase approximations for the commercial and sport catch convolutions does not compensate for the errors introduced by omitting the age 2 fish. In all cases the combined deconvolution performs better than any other index as a measure of recruitment.

For the real data, recruitment estimates obtained by deconvolving the three abundance indices appear more variable than the abundance indices, as expected (Fig. 3a, 3b). Because the convolution weighting function for commercial catch is dominated by a single term, this recruitment estimate most closely resembles its abundance series. Because we do not know the actual value of recruitment, we can evaluate the estimates only by examining their consistency and comparing it with that of the simulations. The three recruitment estimates are only moderately correlated with each other (0.47, 0.42, and 0.35; Table 3) compared with similar correlations among the estimates for simulated data (0.61, 0.58, and 0.50; Table 3). This is due in part to the lower values of correlation among the abundance indices for the real data (0.47, 0.50, and 0.31; Table 3)

TABLE 3. Correlations between recruitment indices. Correlations in the lower triangle are from the observed data and correlations in the upper triangle are from Monte Carlo simulations with observational and process errors. Indices included in the table are (1) commercial CPUE, (2) sport CPUE, (3) adult spawners; (4) deconvolved commercial catch, (5) deconvolved sport catch, (6) deconvolved adult spawners, and (7) combined deconvolution.

Index	1	2	3	4	5	6	7
1	—	0.64	0.65	0.95	0.58	0.56	0.87
2	0.47	—	0.54	0.61	0.88	0.50	0.70
3	0.50	0.31	—	0.51	0.44	0.77	0.69
4	0.92	0.50	0.39	—	0.61	0.58	0.92
5	0.37	0.85	0.26	0.47	—	0.50	0.71
6	0.30	0.26	0.75	0.42	0.35	—	0.81
7	0.72	0.60	0.66	0.85	0.63	0.77	—

as opposed to the simulated data (0.64, 0.65, and 0.54; Table 3). In the real data, the raw abundance series show some covariation. Deconvolution does not increase the correlations among the three abundance indices in either the real data or the simulations, but it does result in higher correlations with recruitment in the simulations.

The Monte Carlo simulations indicate that the three series most highly correlated with recruitment should be commercial CPUE, deconvolved commercial catch, and the combined deconvolution. These three series do in fact show much similarity in the real salmon data (Fig. 3c). The similarity between these three series results from the stability of the deconvolution of commercial catch and the large contribution that commercial catch makes to the combined abundance index. Even if there were large observational errors in commercial catch, these three series would still be highly correlated. However, because commercial catch is enumerated rather than estimated, this series probably has the smallest observational errors. Consequently, these series probably do provide the best estimates of recruitment to the stock.

Comparison of the correlations between the combined deconvolution and other estimates of recruitment shows similar patterns for the Monte Carlo simulations and the real data (Table 3). In all cases, the correlations from the Monte Carlo simulations are larger in magnitude than the corresponding correlations from the real data. This strongly suggests that the coefficients of variation for observational errors, process errors, or both may be greater than the value of 10% used in the simulations. While it may be possible to draw inferences about the error structure of the real data from the variance–covariance matrices of all the time series in observed and simulated data sets, such inferences would be very weak, since there are no replicates for the observed data series. Nevertheless, the simulation results are consistent with the conclusion that the deconvolution of the combined abundance index provides a more accurate description of recruitment to the Central Valley stock of chinook salmon than any of the other indices examined.

Conclusions

When attempting to detect relationships between environmental variables and recruitment when only abundance data are present, two options are available. Convolution of the environment restores the correlation between the environment and recruitment, but increases the variance of estimates of the correlation to the point where this option is not worthwhile. Decon-

volution of abundance series, on the other hand, can provide very reasonable recruitment estimates when the deconvolutions are stable and errors are relatively small. This option restores the correlation between the environment and recruitment without affecting the variance of calculated correlation coefficients. However, because deconvolution amplifies errors to some extent, large observational or process errors can adversely affect deconvolutions to the point where raw abundance data are a better index of recruitment than the deconvolved abundance. When several abundance series are available, recruitment estimates may be improved by combining the abundance indices and deconvolving them collectively.

In the Central Valley stock of chinook salmon, the catch and spawning escapement series are reasonably good indices of recruitment because they are composed of few age-classes and are dominated by the 3-yr-old age-class. In populations with more age structure, abundance indices like catch or spawning stock would not perform as well in providing a reasonable record of recruitment. On the other hand, since more age structure requires that each cohort makes a smaller relative contribution to total abundance, deconvolution of the abundance series would be more sensitive to observational errors. Consequently, there is a trade-off between the loss of information that occurs when abundance data are used to represent recruitment and the amplification of errors through deconvolution of the abundance data. Any investigation that includes deconvolution should, therefore, also include an evaluation of the relative performance of abundance data and the deconvolution of abundance data.

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Appendix

Deconvolution

Convolution matrices were calculated as follows:

Commercial catch:

$$\text{(A1)}\quad C_{1,a,t} = N_{a,t}\,\frac{F_{1,a,t}}{Z_{a,t}} \times [1 - P_a\exp(-Z_{a,t}t_1) - (1-P_a)\exp(-Z_{a,t}t_2)]$$

Sport catch:

$$\text{(A2)}\quad C_{2,a,t} = N_{a,t}\,\frac{F_{2,a,t}}{Z_{a,t}} \times [1 - P_a\exp(-Z_{a,t}t_1) - (1-P_a)\exp(-Z_{a,t}t_2)]$$

Spawning escapement:

$$\text{(A3)}\quad S_{a,t} = P_a N_{a,t}\exp(-Z_{a,t}t_1)$$

survival of a cohort to the next year:

$$\text{(A4)}\quad N_{a+1,t+1} = N_{a,t}(1-P_a)\exp[-Z_{a,t}t_2 - M(1-t_2)]$$

where

a = age ranging from 2 to 5
t = time ranging from 1 to 25
$C_{1,a,t}$ = commercial catch of age a fish in year t

TABLE A1. Fishery and population data for Central Valley chinook salmon. Commercial effort is reported in thousands of deliveries, sport effort in thousands of angler days, and catches and escapements in thousands of fish.

t	Year	Commercial effort ($f_{1,t}$)	Sport effort ($f_{2,t}$)	Commercial catch	Sport catch	Total spawners	Adult spawners
1	1962	33.1	172.2	203.8	97.7	257.0	214.0
2	1963	37.7	115.3	330.0	72.6	301.4	284.1
3	1964	37.6	114.7	276.9	85.2	322.3	264.1
4	1965	43.7	122.7	332.4	51.2	198.2	175.1
5	1966	38.0	144.9	163.2	67.1	197.1	185.4
6	1967	38.2	160.7	87.5	66.2	181.6	146.8
7	1968	44.5	185.0	226.2	148.9	209.5	177.5
8	1969	43.7	183.2	280.4	128.3	342.0	289.4
9	1970	43.7	226.5	226.8	111.1	316.4	245.3
10	1971	38.6	275.0	150.7	166.3	313.9	254.1
11	1972	37.8	234.5	229.8	187.6	204.2	145.4
12	1973	55.6	245.8	422.7	180.9	300.0	251.2
13	1974	51.1	255.8	282.7	141.6	286.3	245.7
14	1975	43.0	173.4	234.4	92.7	269.1	220.7
15	1976	60.0	187.2	237.9	68.6	231.3	199.0
16	1977	61.5	215.0	263.9	77.3	240.3	195.8
17	1978	66.8	176.1	291.0	68.2	172.3	151.2
18	1979	47.8	173.4	233.3	96.3	245.9	183.6
19	1980	50.5	142.1	282.1	76.6	230.5	194.2
20	1981	47.8	128.0	289.9	72.5	343.0	261.2
21	1982	57.7	170.4	418.4	128.3	284.4	222.4
22	1983	30.4	111.6	178.2	52.3	253.5	154.7
23	1984	25.0	123.6	221.7	79.5	310.1	238.9
24	1985	32.4	185.6	205.9	111.4	373.0	314.5
25	1986	60.4	181.7	466.6	111.2	303.6	259.9

TABLE A2. Age-specific population life-history parameters estimated by separable virtual population analysis (Kope 1987).

	Age (a)			
	2	3	4	5
Commercial vulnerability ($q_{1,a}$)	0.000125	0.027676	0.028027	0.028739
Sport vulnerability ($q_{2,a}$)	0.000449	0.002082	0.000947	0.001081
Spawning probability (P_a)	0.049987	0.411060	0.933543	1.000000

$C_{2,a,t}$ = sport catch of age a fish in year t
$S_{a,t}$ = age a spawners in year t
$N_{a,t}$ = abundance of age a fish in year t
P_a = probability of maturation at age a
$F_{1,a,t}$ = commercial fishing mortality rate = $q_{1,a}f_{1,t}$
$F_{2,a,t}$ = sport fishing mortality rate = $q_{2,a}f_{2,t}$
$q_{1,a}$ = vulnerability to commercial fishery at age a
$q_{2,a}$ = vulnerability to sport fishery at age a
$f_{1,t}$ = commercial effort in year t
$f_{2,t}$ = sport effort in year t
$Z_{a,t}$ = total mortality rate = $m + F_{1,a,t} + F_{2,a,t}$
m = natural mortality rate = $0.2 \cdot \text{yr}^{-1}$
t_1 = time from beginning of fishing season to maturation of spawners = 1/3 yr
t_2 = length of fishing season = 1/2 yr.

We calculated nonstationary convolution coefficients for each abundance index by substituting observed fishing efforts (Table A1) and estimates of age-specific vulnerabilities and spawning probabilities (Table A2) into equations (A1) through (A4) with boundary conditions specified by setting recruitment $(N_{2,t}) = 1.0$ for $t = 1$ to 25 and calculating $N_{a,1}$ for $a = 3$ to 5 using average commercial and sport fishing effort in equation (A4). We placed these coefficients in matrix form to obtain the convolution matrices ($\tilde{\boldsymbol{B}}$) by letting

$$\text{(A5)} \quad \tilde{b}_{ij} = C_{1,\,j+t-a+5,i+t-1}$$

for the commercial catch,

$$\text{(A6)} \quad \tilde{b}_{ij} = C_{2,j+t-a+5,i+t-1}$$

for the sport catch, and

$$\text{(A7)} \quad \tilde{b}_{ij} = S_{j+t-a+5,i+t-1}$$

for spawning escapement. Because the convolutions were mixed phase, $\boldsymbol{B}$ was obtained by truncating two columns from the left side of $\tilde{\boldsymbol{B}}$ and one column from the right side of $\tilde{\boldsymbol{B}}$. Consequently, after adjusting the elements of $\boldsymbol{B}$ with equation (13), deconvolution by equation (14) produced recruitment estimates ($\hat{x}_t$) for $t = 0, 1, \ldots, n-1$.

We performed all computations on a Zenith® ZW-158 personal computer without a math coprocessor using programs

written and compiled in Borland International Turbo Pascal®, version 3.01A. This provided 6 byte real numbers and 2 byte integers.

Monte Carlo Simulations

For the Monte Carlo simulations, pseudo-random series of recruitment ($N_{2,t}$: $t = -2$ to 25) were generated from a normal distribution with a coefficient of variation of 0.25. Pseudo-random normal deviates were generated using Box and Muller's (1958) transformation of uniform random variables from Turbo Pascal's random number generator. This approximated the average variability in recruitment estimates obtained by deconvolution of the real data. Normally distributed pseudo-random series of commercial fishing effort ($f_{1,t}$: $t = -2$ to 25) and sport fishing effort ($f_{2,t}$: t −2 to 25) were generated with a means and standard deviations estimated from the observed data (Table A1). Artificial catch and spawning escapement (for years $t = 1$ to 25) were then generated with equations (A1) through (A4) using estimates of age-specific vulnerabilities and spawning probabilities (Table A2). Observational errors were approximated by multiplying the artificial abundance estimates by normally distributed independent pseudo-random numbers with mean 1.0 and variance 0.1 prior to deconvolution, and process errors were simulated by multiplying the individual elements of the convolution matrices ($\bar{B}$) by normally distributed independent pseudo-random numbers with mean 1.0 and variance 0.1 when recruitment was convolved to generate abundance indices. The artificial abundance indices were then deconvolved as described above for the real data.

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Dynamics of Lake Michigan Phytoplankton: Relationship to Nitrogen and Silica Fluxes[1]

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Laird, G. A., D. Scavia, G. L. Fahnenstiel, L. A. Strong, and G. A. Lang. 1988. Dynamics of Lake Michigan phytoplankton: relationship to nitrogen and silica fluxes. Can. J. Fish. Aquat. Sci. 45: 1459–1466.

We describe rates of nitrogen and silica concentration change during May–August 1983 and 1984 in southeastern Lake Michigan and compare those rates with measured plankton processes. Epilimnetic dissolved inorganic nitrogen depletion, compared with total phytoplankton demand, suggests that about 44% of the nitrogen demand is recycled. Epilimnetic diatom production, calculated from soluble silica depletion, is a relatively small fraction (<20%) of total primary production, even when diatoms dominate. Sedimentation of epilimnetic diatom silica, compared with total silica depletion, suggests that a large portion (approximately 43%) of epilimnetic particulate silica sedimentation is due to nonliving diatoms.

Les auteurs décrivent la variation des concentrations d'azote et de silice notées de mai à août 1983 et 1984 dans la partie sud-est du lac Michigan et la comparent aux taux mesurés du processus planctonique. Le taux de disparition de l'azote inorganique dissous de l'épilimnion, comparé à la demande totale du phytoplancton, porte à croire que 44 % environ de l'azote est recyclé. La production de diatomées de l'épilimnion, calculée à partir de la disparition de la silice soluble, représente une fraction relativement faible (<20 %) de la production primaire totale, ceci même lorsque les diatomées dominent. La comparaison de la sédimentation de la silice des diatomées de l'épilimnion et de la disparition de la silice totale porte à croire qu'une grande partie (43 % environ) de la sédimentation de la silice particulaire de l'épilimnion est constituée de diatomées mortes.

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We have been studying the dynamics of southern Lake Michigan plankton with particular emphasis on assemblage growth rates, carbon (C) flow, and trophic interactions. Herein we report rates of change of silica (Si) and nitrogen (N) concentrations during 1983 and 1984 and use these rates to estimate diatom production and Si sedimentation. We also combine these and other data to attempt an epilimnetic Si balance for the period of May to early July and to evaluate the relative contribution of new versus recycled production with respect to N supply.

Other biological, chemical, and physical properties, determined in the region of the 100-m depth contour in southeastern Lake Michigan off Grand Haven, Michigan, between May and August 1983 and 1984, include phytoplankton and zooplankton species composition and vertical structure, primary production, phytoplankton and particulate organic C sedimentation, zooplankton grazing, effects of fish predation, bacteria production and dynamics, and water movements. These data have been evaluated and reported elsewhere (Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987a, 1987b, 1987c; Scavia et al. 1986a, 1986b, 1988; Laird et al. 1986, 1987; Gardner et al. 1986, 1987; Scavia and Laird 1987; McCormick et al. 1985; J. H. Saylor, unpubl. data). This collection of studies represents the most recent evaluation of plankton dynamics and controls in what has become a rather long but sparse history of such studies of Lake Michigan (e.g. Bartone and Schelske 1982; Parker et al. 1977a, 1977b; Conway et al. 1977; Schelske et al. 1974, 1984; Schelske 1975, 1985; Stoermer et al. 1978; Glover 1982; Eadie et al. 1984). Our focus here, on N and Si dynamics, is designed to combine these in vitro observations with the primarily in situ information of the previous studies.

Methods

Water samples were taken from various depths in 5-L opaque Niskin bottles aboard the R/V *Shenehon* between May and August of 1983 and 1984. Sample locations were determined following a Lagrangian scheme in whch parcels of water were tracked with a satellite-monitored drogue system (Pickett et al. 1983) deployed in the vicinity of the 100-m depth contour, 26 km west of Grand Haven, Michigan (43°1′11″N, 86°36′48″W). These windowshade drogues (Scavia and Fahnenstiel 1987) were 1 m wide and of variable length, set to the depth of the mixing layer (typically 10 m). The drifter buoy was tracked via the Argos satellite system (Pickett et al. 1983) and drifter location and hull temperature were captured, at best, every 4 h, but usually every 8–12 h. The drogue was retrieved and released four times in 1983 and seven times in 1984.

Biogenic Si was determined with material collected on 47-mm, 0.4-μm Nuclepore filters following a wet alkaline extraction (Krausse et al. 1983). Soluble reactive Si was determined with an automated procedure (Technicon Method No.

[1]GLERL Contribution No. 554.

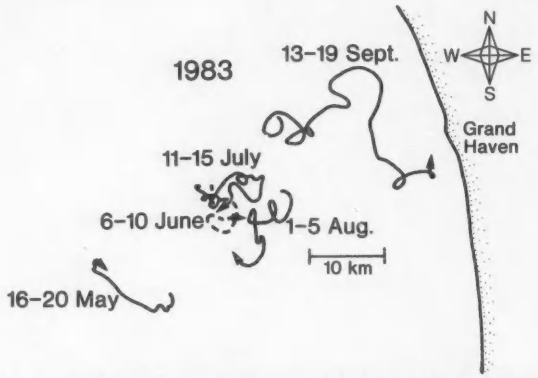

FIG. 1. Tracks of drogue locations recorded during 1983 deployments in the vicinity of the 100-m depth contour, 26 km west of Grand Haven, Michigan.

105-71w/tentative 1972, Technicon Industrial Systems, Technicon Instruments Corporation, Tarrytown, NY) on water passing a 0.2-μm Nuclepore filter. Size-fractioned samples taken from the surface water at the same station in 1987 (D. J. Conley and D. Scavia, unpubl. data) indicate that the concentration of biogenic Si between 0.2 and 0.4 μm increased slowly over the season (mean = 0.34 μM, SE = 0.072) and constitutes a significant fraction (approximately 10–20%) of total biogenic Si only after the larger particles have sedimented from the epilimnion.

Si and C contained in living diatoms were calculated from cell counts and volume estimates converted with equations developed for diatom C (Strathmann 1966) and for freshwater diatom Si: $\log_{10}(\text{Si}) = (1.03 \pm 0.06)\cdot\log_{10}(V) - (2.45 \pm 0.19)$ with Si in picomoles per cell and V in cubic micrometres per cell ($R^2 = 0.82$, $p < 0.0001$, $N = 62$; Conley et al. 1988).

Nitrate was measured by copper–cadmium (Cu-Cd) reduction following an automated procedure (Technicon Method No. 158-71w, 1972, Technicon Industrial Systems, Technicon Instruments Corporation, Tarrytown, NY; Malczyk and Eadie 1980) and ammonium was determined by a microfluorometric method based on *o*-phthaladehyde derivation (Gardner 1978; Gardner and Miller 1981).

Nutrient depletion rates were calculated from linear regression of depth-averaged concentration on time. Regression confidence is reported as standard errors of the determined slopes.

Results

Water Movements

The most persistent feature of our drogue tracks were inertial-period circles (17-h period, average diameter 2.8 km) and longer-term direction reversals (Fig. 1), like the "meandering flow" described for similar drogues in offshore autumn Lake Michigan water (Pickett et al. 1983). The net direction of drogue movement in our experiments varied throughout the summer, as well as during each deployment (Table 1). Drogue net speeds for each deployment ranged between 0.0023 and 0.061 $m\cdot s^{-1}$ between May and September 1983 and 1984 (mean ± SD = 0.028 ± 0.018 $m\cdot s^{-1}$) and were similar to those measured in 1984 in the same region with vector-averaging current meters (Table 1). These measurements indicate that in offshore surface waters during summer, advective transport was slow.

Short-Term Variability

To explore short-term (weekly) versus longer-term (monthly) changes in nutrient concentration, we sampled 4 d apart at drogue locations on each monthly cruise in 1983. Because the drogues track near-surface currents in the Great Lakes under moderate winds and waves (McCormick et al. 1985), changes in nutrient concentrations observed over this time period should be due to biological and chemical alterations and not to changes in water masses.

During the 4-d cruise in May, temperature remained uniform vertically and temporally and we detected no significant changes in N or Si concentrations. While diatoms were certainly growing during this period (Fahnenstiel and Scavia 1987a), vertical mixing apparently minimized their impact on water-column-averaged nutrient concentrations over the 4 d. However, with the onset of weak thermal stratification in June, soluble Si concentrations decreased measurably during the 4-d interval through the top 30 m (Fig. 2). Concentration change during this period was consistent with the longer-term depletion rate (see two June data points along regression line in Fig. 6a below). No notable changes occurred during each of the 4-d experiments in July and August, except that subthermocline ammonium concentrations increased (Fig. 2), consistent with the seasonal trend described below (Fig. 3b).

Seasonal Trends

The water column at our 100-m station stratified thermally in early June 1983 and late June 1984 and, once stratified, thermocline depths varied between 10 and 15 m (Laird et al. 1987).

TABLE 1. Drifter movement.

	1983			1984			1984	
	Speed ($m\cdot s^{-1}$)	Direction (°)	Time (d)	Speed ($m\cdot s^{-1}$)	Direction (°)	Time (d)	Speed ($m\cdot s^{-1}$)[a]	Time (d)
May	0.030	297	4	—	—	—	—	—
June	0.021	127	4	0.002	38	14	0.013 ± 0.007	16
July	0.020	82	4	0.009	90	3	0.005 ± 0.003	5
				0.019	7	2	0.015 ± 0.010	5
August	0.030	215	4	0.061	60	1	0.043 ± 0.017	5
				0.044	237	5	0.027 ± 0.025	5
September	0.044	86	6	—	—	—	—	—

[a]G.S. Miller, GLERL/NOAA, unpubl. data; mean ± SD of four current meters.

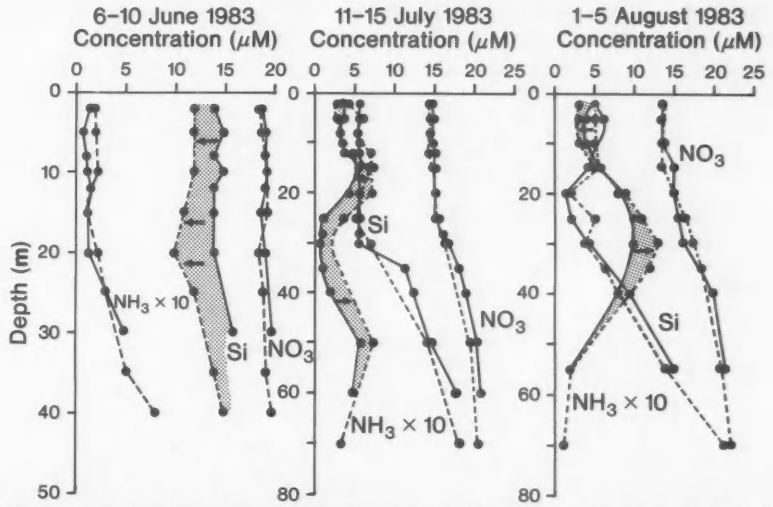

FIG. 2. Vertical profiles of ammonium, soluble Si, and nitrate plus nitrite concentrations at the beginning and end of 4-d periods at drogue locations during June, July, and August 1983. Solid line represents the first day and the broken line represents the last day of each 4-d sequence.

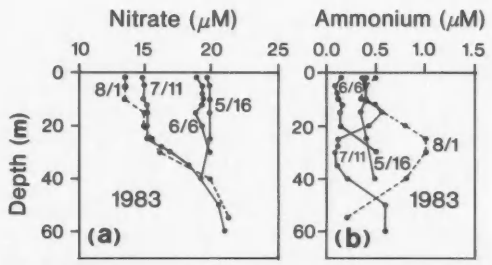

FIG. 3. (a) Nitrate plus nitrite and (b) ammonium concentration vertical profiles for 1983.

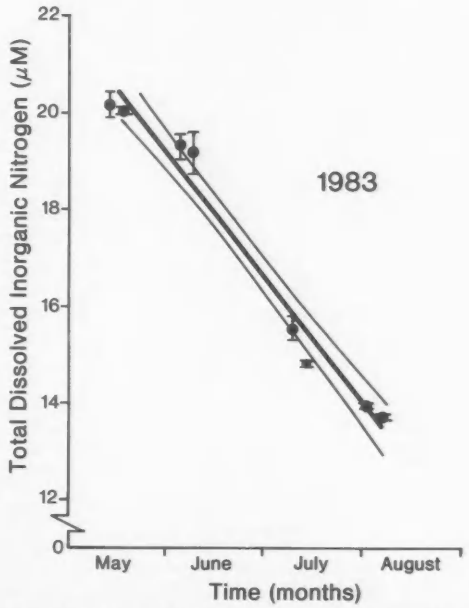

FIG. 4. Epilimnetic total dissolved inorganic N concentration versus time in 1983. Vertical bars represent ±1 SD of data from two to five depths within the epilimnion on each date. Lines represent regression results with 95% confidence intervals.

Prior to stratification, ammonium and nitrate plus nitrite (referred to here as nitrate) concentrations were uniform with depth. Surface nitrate concentration (Fig. 3a) decreased between 6 June and 11 July 1983 and thereafter remained low in the epilimnion, increased with depth through the region of the deep chlorophyll layer (DCL) (25–50 m; Fahnenstiel and Scavia 1987c), and increased slowly with time deeper in the hypolimnion. Ammonium concentrations (Fig. 3b) decreased in the epilimnion and DCL regions (20–35 m) between 16 May and 6 June 1983, increased in the epilimnion and decreased in the DCL (25–50 m) between 6 June and 11 July, and then increased in the upper portion of the DCL between 11 July and 1 August. Epilimnetic total inorganic N depletion rate for the period of late May to early August 1983 was 0.088 $\mu M \cdot d^{-1}$ (SE = 0.0063, $R^2 = 0.97$, $p < 0.00001$; Fig. 4).

Soluble Si concentrations initially were similar in both years (Fig. 5). Concentrations in the surface waters decreased between 16 May and 11 July 1983 at a rate of 0.21 ± 0.014 $\mu M \cdot d^{-1}$ ($R^2 = 0.98$, $p < 0.0001$; Fig. 6a) and between 21 May and 5 July 1984 at 0.25 ± 0.013 $\mu M \cdot d^{-1}$ ($R^2 = 0.96$, $p < 0.001$; Fig. 6b). Concentrations in deeper water decreased prior to the onset of thermal stratification and then remained fairly constant through the remainder of the season. While initial concentrations and seasonal depletion rates were similar for both years, soluble Si concentration decreased to values lower in 1984 compared with 1983.

Epilimnetic biogenic Si concentration in 1984 was highest on 21 May (8.0 μM) and decreased steadily at a rate of 0.100 ± 0.016 $\mu M \cdot d^{-1}$ ($R^2 = 0.95$, $p < 0.03$) to 0.36 μM on 24 August. Concentrations in the region below the thermocline (20–30 m) first increased and then decreased as summer progressed. The epilimnetic total Si (soluble reactive plus bio-

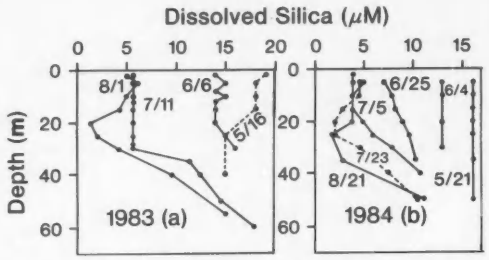

FIG. 5. Dissolved Si concentration vertical profiles for (a) 1983 and (b) 1984.

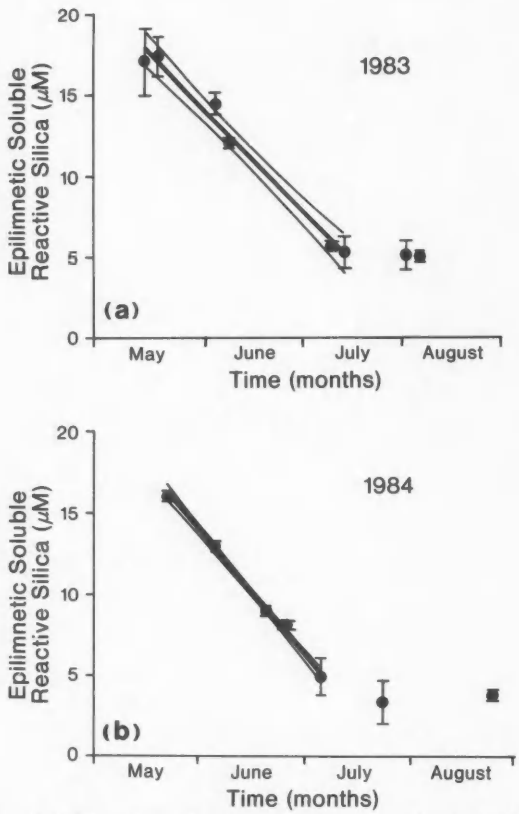

FIG. 6. Epilimnetic soluble Si concentration versus time in (a) 1983 and (b) 1984. See legend to Fig. 4 for details.

genic) depletion rate was 0.35 ± 0.02 μM·d^{-1} (R^2 = 0.99, p < 0.004; Fig. 7) between 21 May and 4 July.

Discussion

Abiotic Factors

Because there was little concentration change during each of the 4-d experiments in 1983 and because those changes were consistent with long-term dynamics, we conclude that our observed concentration changes were driven by seasonal processes. However, before proceeding with analysis of seasonal trends, we must determine whether concentration

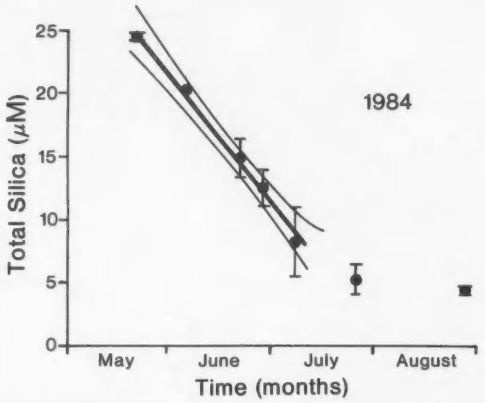

FIG. 7. Total epilimnetic Si concentration versus time in 1984. See legend to Fig. 4 for details.

changes due to abiotic factors are subordinate to biotic processes on that time scale.

Of the potential abiotic factors, horizontal advection is a major concern. Water mass movements, inferred from drogue trajectories and current meters (Table 1), were relatively slow during the period of stratification in offshore waters. These relatively slow speeds become even less important if concentrations show small horizontal gradients because it is the product of these two properties that influences concentration changes at one location. We suggest that this was true for our study. Specific rates of change of chlorophyll concentrations, based on water-mass movements and measured horizontal chlorophyll gradients, were typically ±0.01·d^{-1} during this study (Scavia and Fahnenstiel 1987). Because these values are over an order of magnitude smaller than measured algal growth, grazing, and sinking rates (0.1–0.4·d^{-1}; Fahnenstiel and Scavia 1987a, 1987b, 1987c; Scavia and Fahnenstiel 1987), biological process rates are far greater than horizontal physical transport of algal chlorophyll. Analogously, we conclude that changes in nutrient concentration due to horizontal water movement are not a major concern in summer offshore Lake Michigan waters.

Two other abiotic factors that can influence nutrient dynamics are vertical flux and external loads. Throughout late spring and summer, the presence of an actively growing subthermocline algal assemble (Fahnenstiel and Scavia 1987c) assures that nutrients from the hypolimnion do not reach the epilimnion. This is reflected in the vertical profiles of nitrate and dissolved Si (Fig. 3a, 5) that show depletion well below the depth of the thermocline (10–15 m), indicating that nutrients from the hypolimnion are sequestered by the subthermocline assemblage. Nutrient budget and model analyses (e.g. Scavia 1980; Stadelmann and Fraser 1974) have shown that dilution by these massive water bodies lends significance to external nutrient loads only on annual time scales; internal cycling drives seasonal dynamics.

Seasonal Dynamics

Because abiotic factors are minimized and short-term variation is small compared with seasonal changes, we now focus on seasonal biotic factors. In 1983, nitrate (Fig. 3a) and soluble reactive Si (Fig. 5a) concentrations were highest early in the year and epilimnion values decreased after the onset of stratification. Nitrate and soluble Si depletion was not confined to

the epilimnion, but rather extended through the metalimnion into the DCL region (20–35 m; Fahnenstiel and Scavia 1987c). Later in summer, when the DCL broadened and deepened (25–50 m), the zone of nutrient depletion extended only into the upper portion of the DCL. This finding is consistent with reports of relatively fast algal growth in the upper portions of the DCL and light-limited slower rates in the lower portions (Fahnenstiel et al. 1984; Fahnenstiel and Scavia 1987a, 1987b) and with the fact that nitrate and Si are only slowly regenerated in the water column.

Ammonium is the primary form of recycled N and therefore its dynamics closely reflect the interaction of plankton demand and community regeneration. Decreasing ammonium concentration between 16 May and 6 June 1983 suggests that, during that time, demand exceeded supply rates. As epilimnetic phytoplankton production and abundance decreased later in the season (Scavia and Fahnenstiel 1987), ammonium concentration increased, indicating that regeneration rate was greater than assimilation. However, during that time, ammonium concentrations associated with the upper portion of the DCL (25–35 m) continued to decrease (Fig. 3b) and the balance was still shifted toward demand. In August, as the DCL deepened further (30–60 m), the zone of ammonium increase above the DCL deepened and broadened, indicating a region of dynamic nutrient regeneration. The fact that highest concentrations of crustacean zooplankton were also found in that same region (J. T. Lehman and D. Scavia, unpubl. data) suggests that these crustaceans may be the primary source of regenerated N on weekly to monthly time scales (see below).

Si depletion, while proceeding at similar epilimnetic rates in 1983 and 1984, resulted in slightly different vertical concentration profiles in those years (Fig. 5). While concentrations in the top 20 m were initially similar in both years, they were lower in July and August 1984 (3.96 ± 0.22 μM, $N = 13$) compared with 1983 (5.21 ± 0.29 μM, $N = 22$). Concentrations were similar in the upper region of the DCL in both years, but lower at 40 m (7.5 vs. 11.0 μM) and 50 m (10.7 vs. 14.8 μM) in 1984 compared with 1983. We suggest that the difference between the two midsummer vertical profiles is linked to the delayed thermal stratification in 1984 (Scavia and Fahnenstiel 1987) that prolonged effective mixing of diatom production and subsequent Si depletion throughout the water column. Shortly after the onset of thermal stratification, the Si-limited diatoms cannot combat sedimentation losses and are selectively removed from the epilimnion (Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987b), thus reducing the Si demand and depletion rates there. While production below the thermocline can be significant (approximately 50% of the water column rate; Fahnenstiel and Scavia 1987b, 1987c), its rate is lower than in spring and is confined to the 15- to 25-m depth range. Thus, the onset of thermal stratification reduces Si depletion throughout the water column and it is likely that delayed stratification in 1984 allowed total water column Si depletion to proceed longer into the season resulting in lower summer concentrations.

Implications for Plankton Fluxes

Because the dominant nutrient concentration changes during our study were due to biological processes acting on seasonal time scales, it is possible to relate those observed rates of change to estimates of plankton processes. Specifically, we make comparisons of soluble inorganic N depletion with phytoplankton N demand, soluble Si depletion with diatom Si demand, and total (soluble plus biogenic) Si depletion with diatom sedimentation.

N cycling

Here, we compare the rate of change of epilimnetic dissolved inorganic N (nitrate + nitrite + ammonium) with phytoplankton demand, calculated from net phytoplankton carbon production. If net inorganic N depletion is due to phytoplankton assimilation only, then phytoplankton N demand, predicted from algal C production and a C:N ratio, will be similar to the observed N depletion rate; any difference between measured and predicted rates should estimate the N supply rate.

Total inorganic N depletion rate for the period from late May to early August 1983 was 0.088 ± 0.006 $\mu M \cdot d^{-1}$ (Fig. 4). Autotrophic C production, measured by 24-h in situ ^{14}C incubations in 2-L bottles (Fahnenstiel and Scavia 1987a) integrated over the same depth and time period, was 1.13 $\mu M\ C \cdot d^{-1}$ (Scavia and Fahnenstiel 1987). Assuming a C:N atom ratio of 7.2 (mean of 8.1, Brzezinski 1985; 6.6 "Redfield ratio"; 7.2, Parsons et al. 1961; 7.0, Strickland 1960) yields a N demand of 0.157 $\mu M \cdot d^{-1}$ and a required N supply rate of 0.069 $\mu M \cdot d^{-1}$. This represents 44% of the algal demand, illustrating that, like in other systems (e.g. Stadelmann and Fraser 1974; Eppley and Peterson 1979), much of the N assimilated by phytoplankton is recycled.

Crustacean zooplankton are one source of this recycled N (Lehman 1980; Bidigare 1983). Normalizing our calculated N supply rate by average epilimnetic crustacean zooplankton biomass (J. T. Lehman and D. Scavia, unpubl. data; Scavia and Fahnenstiel 1987) yields a weight-specific rate of 16.4 μg N·mg dry weight^{-1}·d^{-1}, a rate similar to those determined directly in other field and laboratory experiments with crustaceans (5.1–27.9, mean = 15.5, SE = 3.2, $N = 6$; Wetzel 1983).

The above analysis considers only Lake Michigan's traditional food web (nano- and microplanktonic autotrophs and crustacean zooplankton) as major mediators of N flux on weekly to monthly time scales. We have recently documented Lake Michigan heterotrophic bacterioplankton production that rivals autotrophic production (Scavia et al. 1986b; Scavia and Laird 1987) and micrograzer bacterivory that apparently often keeps pace with the rapid bacterial growth rates (Gardner et al. 1986, Scavia and Laird 1987), yielding little change in bacteria biomass over the season. The microbial food web also has the apparent capability of processing added amino acids and altering N fluxes in relatively long incubations (Gardner et al. 1987); however, the actual roles of bacteria and their grazers in controlling inorganic N concentration over days to months in nature are not clear. We assume here that N–bacteria–micrograzer dynamics (driven by typical bacterial turnover times of 5–25 h; Scavia and Laird 1987) are in dynamic equilibrium with respect to the longer time frame of the traditional web (driven by typical phytoplankton turnover times of 2–10 d; Fahnenstiel and Scavia 1987a) and therefore they do not strongly influence our conclusions. Work will have to be done to further test this assumption.

Diatom production

Our epilimnetic dissolved Si depletion rates (0.21 $\mu M \cdot d^{-1}$ for 16 May to 14 July 1983 and 0.25 $\mu M \cdot d^{-1}$ for 21 May to 5 July 1984; Fig. 6) are similar to others (0.19 $\mu M \cdot d^{-1}$, assuming 60 d between the beginning of the spring diatom bloom and stratification, Conway et al. 1977; 0.3 $\mu M \cdot d^{-1}$, Bartone and Schelske 1982) and can be used to estimate average epilimnetic diatom production during the period from late May to late July

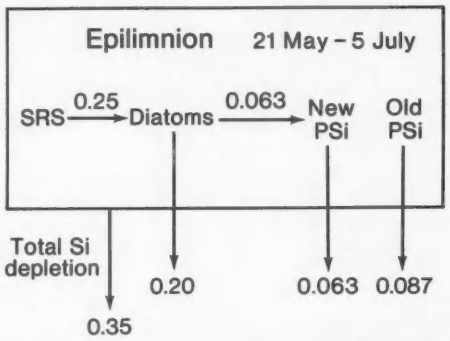

FIG. 8. Epilimnetic Si balance for the spring–summer period in 1984. SRS = soluble reactive Si; PSi = particulate Si of biogenic origin. All rates are micromoles per litre per day. Diatom Si decreased at a rate of 0.013 $\mu M \cdot d^{-1}$.

1983 and 1984. We estimated Si:C ratios for diatoms in epilimnetic water samples collected between May and July of 1983 and 1984 by calculating for each date and depth the ratio of total diatom Si to total diatom C, each determined by conversion from cell counts and measured cell volumes using the equations of Strathmann (1966) for C and Conley et al. (1988) for Si. The mean atom ratio was 0.75 (SE = 0.08, N = 24). Dividing this ratio into the dissolved Si depletion rate yields diatom C production estimates of 0.28 $\mu M \cdot d^{-1}$ for 1983 and 0.33 $\mu M \cdot d^{-1}$ for 1984. Epilimnetic total phytoplankton production during the same time period ranged between 0.81 and 1.76 $\mu M \cdot d^{-1}$ (integrated mean = 1.33) in 1983 and between 1.13 and 3.32 $\mu M \cdot d^{-1}$ (integrated mean = 2.31) in 1984 (Scavia and Fahnenstiel 1987). The Si-based rate estimates suggest that even when diatoms make up most of the phytoplankton mass, their lower growth rates result in a disproportionately lower fraction of total epilimnetic C production (21% in 1983, 14% in 1984).

This conclusion is supported by comparison of N demand by the entire algal assemblage with that of diatoms only. The diatom Si:N atom ratio, estimated from our Si:C estimate (0.75) and a C:N estimate of 7.2 (see above), is 5.4. Dividing this ratio into the 1983 soluble Si depletion rate yields diatom N demand of 0.039 $\mu M \cdot d^{-1}$. Because net N depletion (0.088 ± 0.006 $\mu M \cdot d^{-1}$; Fig. 4) includes both assimilation and regeneration, 0.088 $\mu M \cdot d^{-1}$ is clearly an underestimate of the total demand; yet the calculated diatom demand is only 44% of even that rate.

These estimates of low diatom production require a slight modification of the interpretation of sedimentation and grazing fluxes determined from the same study but reported earlier. Scavia and Fahnenstiel (1987) suggested that sedimentation is important in controlling diatom *spring* abundance and that *summer* epilimnetic production rates are within 30% of measured sinking and grazing loss rates. That analysis suggested further that grazing was not important during spring when diatoms dominate. However, in light of the present analysis, it seems that while the slower growing diatoms were not grazed (diatom production = 0.28–0.33 $\mu M \cdot d^{-1}$; diatom sedimentation = 0.25 $\mu M \cdot d^{-1}$, from integrated sequential trap deployments 23 May to 5 July 1984; Scavia and Fahnenstiel 1987), nondiatom production likely was grazed. If true, grazing could explain the large discrepancy between early spring (May to early June) total algal production (2–3 $\mu M \cdot d^{-1}$; Fahnenstiel and Scavia 1987b) and measured loss rates (0.4–0.8 $\mu M \cdot d^{-1}$; Scavia and Fahnenstiel 1987), the latter attributed almost exclusively to sedimentation. The method of determining grazing loss rates in those experiments was based on measuring chlorophyll concentration changes in experimental enclosures (Scavia and Fahnenstiel 1987), and during spring, the high abundance of relatively ungrazed diatoms may have masked the effects of zooplankton on the cooccurring flagellate community. Analysis of changes in species abundances from June 1984 grazing experiments confirms that, while diatoms were not grazed significantly at that time, phytoflagellates were (D. Scavia and G. L. Fahnenstiel, unpubl. data).

Diatom sedimentation

The epilimnetic total Si depletion rate (0.35 ± 0.02 $\mu M \cdot d^{-1}$ between 23 May and 5 July 1984; Fig. 7) estimates downward flux of epilimnetic diatom production. Diatom Si flux, determined from counts of trap-collected viable cells and cell size measurements converted to Si, integrated over the same period is 0.20 $\mu M \cdot d^{-1}$. Comparison with total Si loss rate suggests that a substantial portion (43%) of the flux was due to nonliving diatoms. This is consistent with the fact that our calculated diatom Si concentration from epilimnetic samples accounts for only 23–37% of measured biogenic Si. Flux of nonliving diatoms and diatom fragments also contributed substantially to biogenic Si flux in Grand Traverse Bay in northern Lake Michigan (Glover 1982). Nonliving cells and fragments in the surface waters (0–40 m) there accounted for 45.5% of biogenic Si.

Spring–summer Si balance

Compared with sedimentation and production rates, allochthonous Si load is low (approximately 0.002 $\mu M \cdot d^{-1}$; Parker et al. 1977a). Biogenic Si dissolution rate is also slow (approximately 0.007 $\mu M \cdot d^{-1}$), assuming a recycle rate of 90%·yr^{-1} (Conway et al. 1977) and mean particulate Si concentration of 3.0 μM. This means that on a seasonal time scale we can represent epilimnetic Si dynamics as a balance among diatom production, sedimentation, and grazing losses (Fig. 8).

Epilimnetic *diatom* Si (DSi) dynamics can be represented by the following simple model:

$$(1) \quad \Delta DSi/\Delta t = \text{Production} - \text{sedimentation} - \text{grazing}.$$

Comparison of diatom sedimentation (0.20 $\mu M \cdot d^{-1}$) and production (0.25 $\mu M \cdot d^{-1}$) rates demonstrates that over 80% of particulate Si formation rate is accounted for by sediment trap collection of viable cells over the period 21 May to 5 July 1984, a conclusion we came to previously (Scavia and Fahnenstiel 1987). Net rate of change of DSi (left-hand side of equation 1), calculated from the rate of change in diatom concentration between 21 May and 5 July, is -0.013 $\mu M \cdot d^{-1}$. Grazing loss, estimated from the first three terms in equation 1, represents Si flux from diatoms to zooplankton of 0.063 $\mu M \cdot d^{-1}$.

The difference between total Si depletion (0.35 $\mu M \cdot d^{-1}$) and diatom Si sedimentation (0.20 $\mu M \cdot d^{-1}$) represents sedimentation of nonliving diatoms and cell fragments (0.15 $\mu M \cdot d^{-1}$). Because only about 0.063 $\mu M \cdot d^{-1}$ can be accounted for by contemporary production of Si in feces, the remaining 0.087 $\mu M \cdot d^{-1}$ comes from dead cells. One source of these dead cells may be concurrent nonpredatory mortality. The flux of nonliving Si represents approximately 35% of the production rate. While crude, this estimate of nonpredatory diatom mortality is similar to that suggested from a C balance for the entire algal community (Scavia and Fahnenstiel 1987); however, in that pre-

vious balance we were also unable to make a statistically significant argument for the process. It is also likely that the sedimenting material is older Si resuspended during winter and early spring, as Eadie et al. (1984) and Chambers and Eadie (1981) have suggested for other particulate material. The mechanism could be similar to that described for plutonium and its association with autogenetically produced particles (Wahlgren et al. 1980). To see if this is reasonable, we compare the required mass of resuspended Si with that determined from full water column balances calculated by Conway et al. (1977) for the same region. Our estimated epilimnetic flux of nonliving Si is 0.087 $\mu M \cdot d^{-1}$. That flux rate maintained over the 45-d period of our analysis indicates a total concentration initially available for sedimentation of 3.92 μM. If that represents the concentration of resuspended material spread uniformly throughout the 100-m water column prior to stratification, then 392 $mmol \cdot m^{-2}$ would have to have been resuspended. Conway et al. (1977) estimated the winter maximum of reactive plus amorphous Si to be approximately 1.3 $mol \cdot m^{-2}$. That value decreases dramatically during spring and summer due to diatom production and sedimentation, but then increases in fall due to dissolution and resuspension. By November, 90% of the original Si has been returned to the water column, with 15% of it still in particulate form. That 15% represents 195 $mmol \cdot m^{-2}$. If we assume that the rest of the material is resuspended between November and the following spring (total 25%), then the resuspended particulate Si would be 325 $mmol \cdot m^{-2}$, a value not unlike our calculated requirement of 392 $mmol \cdot m^{-2}$; however, nonpredatory mortality may play a role as well.

Conclusion

We have combined nutrient fluxes estimated from changes in concentration to various plankton processes determined in vitro during the same study and come to the following conclusions regarding plankton–nutrient processes in southern Lake Michigan. Epilimnetic dissolved inorganic N depletion, compared with total phytoplankton demand, suggests that about 44% of the N demand is recycled. Eplimnetic diatom production, calculated from soluble Si depletion, is a relatively small fraction (<20%) of total primary production, even when diatoms dominate. Sedimentation of epilimnetic diatom Si, compared with total Si depletion, suggests that a large portion (approximately 43%) of epilimnetic particulate Si sedimentation is due to nonliving diatoms. Some simple budgets suggest further that a large portion of that dead Si is resuspended.

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Influence of Nutrient Enrichment and Light Availability on the Abundance of Aquatic Macrophytes in Florida Streams[1]

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Canfield, D. E. Jr., and M. V. Hoyer. 1988. Influence of nutrient enrichment and light availability on the abundance of aquatic macrophytes in Florida streams. Can. J. Fish. Aquat. Sci. 45: 1467–1472.

A survey of 17 Florida streams was conducted between October 1984 and August 1986 to determine if the abundance of aquatic macrophytes was related to nutrient enrichment. Macrophyte standing crops were not correlated with in-stream total phosphorus or total nitrogen concentrations. Aquatic macrophytes contained less than 2% of the annual nutrient discharge in nearly all streams. Nutrients are, therefore, not considered to be the primary factor regulating the abundance of aquatic macrophytes in most Florida streams. Shading by riparian vegetation seems to be the dominant factor controlling the location and abundance of aquatic macrophytes. Statistical analyses indicated that the potential average and maximum standing crop of aquatic macrophytes in the sampled streams could be estimated by the equations

$$\log (SC_{avg}) = 1.06 - 0.026\ (\%C) \qquad R^2 = 0.93$$
$$\log (SC_{max}) = 1.54 - 0.014\ (\%C) \qquad R^2 = 0.94$$

where SC_{avg} and SC_{max} are the average and maximum standing crop of aquatic macrophytes (kilograms fresh weight per square metre), respectively, and %C is the percent canopy coverage by riparian vegetation.

Entre octobre 1984 et août 1986, on a fait une étude de 17 cours d'eau de la Floride pour déterminer si l'abondance des macrophytes aquatiques était liée à la fertilisation par des substances nutritives. On n'a pas établi de corrélation entre la biomasse des macrophytes et les concentrations d'azote ou de phosphore totaux dans les cours d'eau. Dans presque tous les cours d'eau, les macrophytes aquatiques contenaient moins de 2 % de substances nutritives déversées annuellement. Par conséquent, les substances nutritives ne sont pas considérées comme le facteur primordial régissant l'abondance des macrophytes aquatiques dans la plupart des cours d'eau de la Floride. L'ombre créée par la végétation des rives semble être le facteur dominant influant sur l'emplacement et l'abondance des macrophytes aquatiques. Les analyses statistiques ont montré que la biomasse potentielle moyenne et maximale de macrophytes aquatiques dans les cours d'eau échantillonnés pouvait être estimée à partir des équations suivantes :

$$\log (BM_{moy}) = 1{,}06 - 0{,}026\ (\%\ C) \qquad R^2 = 0{,}93$$
$$\log (BM_{max}) = 1{,}54 - 0{,}014\ (\%\ C) \qquad R^2 = 0{,}94$$

où BM_{moy} et BM_{max} sont respectivement la biomasse moyenne et maximale de macrophytes aquatiques (kilogrammes de poids frais (par mètre carré), et % C est le pourcentage du couvert fourni par la végétation des rives.

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Many Florida streams experience problems with aquatic macrophytes. Major aquatic plant management programs have, therefore, been established to prevent aquatic weed problems (Nelson and Dupes 1985). The growth of aquatic macrophytes and large algae (i.e. *Cladophora* sp.) to nuisance levels in some northern streams has been linked to the deterioration of water quality resulting from eutrophication (Wong and Clark 1976; Wright and McDonnell 1986a, 1986b). This linkage suggests that the control of nutrients will reduce or eliminate aquatic weed problems (Wright and McDonnell 1986b), but other studies suggest that light availability rather than nutrients is the primary factor limiting aquatic macrophytes in streams (Casey and Westlake 1974; Kern-Hansen and Dawson 1978; Dawson and Kern-Hansen 1978, 1979). Consequently, the efficacy of nutrient removal for the control of aquatic macrophytes in streams is unknown and no assurance can currently be given that the control of anthropogenic nutrient inputs will reduce or eliminate aquatic weed problems in Florida streams. Our purpose here is to examine the influence of nutrient enrichment and light availability on the abundance of aquatic macrophytes in Florida streams.

Methods

An intensive 1-yr study of the Little Wekiva River (Seminole County, Florida) and a survey of eight other streams were initiated in October 1984 to assess the relationship between in-stream water quality and the abundance of aquatic macro-

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phytes. The Little Wekiva River was chosen for study because it was thought that excessive growths of aquatic macrophytes were linked to the introduction of treated wastewater from the Altamonte Springs Regional Sewage Treatment Plant (STP). The eight survey streams were chosen to provide a comparison between streams with low and high nutrient concentrations. After October 1985, the study was expanded to include a total of 17 streams. Six streams (Alexander Springs, Econlockhatchee River, Ichetucknee Springs, Rock Springs, Wacissa River, and St. Marks River) were chosen because they have experienced no significant cultural eutrophication. Six streams (Alligator Creek, Little Econlockhatchee River, Mills Creek, Pottsburg Creek, Reedy Creek, and Wekiva River) were chosen because they receive major inputs of nutrients from anthropogenic sources such as wastewater treatment plants. The final four streams (Alafia River, Hillsborough River, Hogtown Creek, and Santa Fe River) were selected because they receive nutrients primarily from naturally occurring phosphatic formations. Spring-fed streams (Alexander Springs, Ichetucknee Springs, Rock Springs, St. Marks River, Little Wekiva River, and Wekiva River) were studied from their origin at the springs to their mouth. The other streams were studied only in specific reaches to eliminate downstream pollution sources.

Water quality sampling stations were established at 29 sites in the Little Wekiva River to measure nutrient inputs from both point and nonpoint sources and to measure changes in in-stream nutrient concentrations along the length of the stream. For the survey streams, water quality samples were collected from three stations (headwater, midstream, and stream mouth). Water quality samples from the Little Wekiva River were collected monthly between October 1984 and December 1985. After December 1985, samples were collected from the Little Wekiva River and the survey streams every 2 mo until January 1987.

Water velocities were measured at each sampling site by use of a Marsh-McBirney model 201m portable current meter and stream discharge was estimated by use of the procedures described by Platts et al. (1983). Water samples were collected from just below the surface at mid-stream in 1-L, acid-cleaned nalgene bottles. Water samples were placed on ice and then transported to the laboratory for analysis. Total phosphorus (TP) concentrations were determined by using the procedures of Murphy and Riley (1962) with a persulfate digestion (Menzel and Corwin 1965). Total nitrogen (TN) concentrations were determined by using a modified Kjeldahl technique (Nelson and Sommers 1975).

Aquatic macrophytes including large algae such as *Cladophora* sp., *Lynbya* sp., and *Chara* sp. were sampled in the Little Wekiva River and the survey streams from one to three times between March 1985 and August 1986. All sampling was done between March and November of each year because this period corresponds to the period of peak plant abundance in Florida's subtropical streams. Aquatic vegetation was sampled by use of boats and divers. Plants were sampled at roughly uniform intervals as the boats were motored at idle speed from 1 to 7 min. The time of movement was adjusted for the length of each stream so that a minimum of 20 stations (maximum of 90 in the Little Wekiva River) were sampled in each stream. A sampling transect was established perpendicular to the main line of stream flow at each station. Stream width was measured by use of either measuring tapes or calibrated range finders. Stream depth, tree canopy coverage, and the above-ground standing crop of aquatic macrophytes were then measured at five equally spaced locations starting from the middle of the stream channel. At each sampling location, a 0.25-m^2 quadrat was randomly dropped and the divers removed all vegetation present within the quadrat. The sampled vegetation was then spun in nylon mesh bags to remove excess water and weighed to the nearest 0.1 kg. Tree canopy coverage was visually estimated as either 0, 50, or 100% cover above each plant sampling site. The five canopy coverage values, the above-ground macrophyte standing crop values, and the water depths were then averaged to provide a mean value for each sampling transect.

Composite plant samples from the streams were analyzed using the methods described by Canfield et al. (1983) to determine average plant tissue nutrient levels. The water content of all macrophytes was assumed to be 95%. The content of TP in the plant tissues ranged from 2.4 to 7.5 mg/g dry weight and TN levels ranged from 12 to 32 mg/g dry weight. Because these values were similar to those found for aquatic macrophytes in other Florida waters (Canfield et al. 1983), mean tissue nutrient levels for TP (3.7 mg/g dry weight) and TN (23 mg/g dry weight) were used with the average above-ground standing crop values expressed as dry weight to determine the total mass of nutrients associated with the aquatic macrophytes in each stream. The annual TP and TN discharge rates for each stream were calculated according to the methods of Platts et al. (1983) for the last downstream station.

Results

The Little Wekiva River begins at the outlet of Lotus Lake (Seminole County, Florida) and flows unimpeded for 17 km to its junction with the Wekiva River. The upper portion (8 km) drains an elevated physiographic region known as the Apopka Hills, a sandhill region that is highly modified by karst processes (Brooks 1981). The dominant geologic formation is the Hawthorne Formation which consists primarily of sand, silty sand, and clay with phosphorite granules. The Little Wekiva River in this region falls from an elevation of 18.6 to 6.1 m mean sea level. The stream channel is narrow (3–9 m) and water depths in 1985 ranged from 0.1 to 0.8 m (mean 0.4 m). The average instantaneous discharge rate prior to the point source discharges was 0.4 m^3/s (range 0.05–1.9 m^3/s) in 1985. Flow in the upper section of the Little Wekiva River, however, was augmented by the Altamonte Springs Regional STP (0.25 m^3/s), the Weathersfield STP (0.01 m^3/s), and the High Acres Citrus Plant (0.27 m^3/s). TP and TN concentrations in the stream prior to the point source discharges averaged 0.09 and 0.87 mg/L, respectively. After the addition of water from the Altamonte Springs STP (TP = 1.6 mg/L, TN = 5.5 mg/L), the Weathersfield STP (TP = 6.6 mg/L, TN = 6.9 mg/L), and the High Acres Citrus Plant (TP = 0.08 mg/L, TN = 0.27 mg/L), average TP and TN concentrations in the Little Wekiva increased to 0.60 and 2.7 mg/L, respectively.

The lower portion (9 km) of the Little Wekiva River flows through the St. Johns River Offset physiographic region (Brooks 1981). The geology of this physiographic region is dominated primarily by estuarine and riverine deposits, but some limestone occurs near the surface. Stream elevation in this section decreases from 6.1 to 4.2 m mean sea level and the stream channel widens greatly (6–50 m). Water depths in 1985 ranged from 0.3 to 2.5 m (average 0.5 m). The average instantaneous discharge rate at the mouth of the Little Wekiva River was 3.2 m^3/s (range 2.2–6.2 m^3/s) during 1985. Flow in the lower section of the Little Wekiva River increased primarily because of groundwater discharge via three major springs: Sanlando Spring

TABLE 1. Mean plant standing crop, percent canopy cover, and stream depth and width for survey streams. *N* is the number of transects sampled for each stream on a given date. Numbers in parentheses are the lengths of the stream sampled.

Stream	Date	*N*	Plant standing crop (kg fresh wt/m²)	Percent canopy cover	Depth (m)	Width (m)
Little Wekiva (17 km)	March 1985	90	1.3	—	0.5	12
	September 1985	32	2.0	51	0.7	17
	August 1986	22	3.3	44	1.0	16
	Mean		2.2	48	0.7	15
Alexander Springs (13 km)	May 1985	30	3.4	—	0.8	47
	November 1985	18	5.3	29	1.0	38
	June 1986	16	4.4	19	0.8	45
	Mean		4.4	24	0.9	43
Ichetucknee Springs (7.5 km)	May 1985	23	4.7	—	1.0	25
	November 1985	9	2.5	24	1.2	21
	June 1986	17	3.7	21	1.2	26
	Mean		3.6	23	1.1	24
Alligator Creek (5.0 km)	June 1985	27	0.5	—	0.5	7
	November 1985	13	0.6	74	0.8	8
	June 1986	20	0.6	45	0.5	7
	Mean		0.6	60	0.6	7
Rock Springs (12 km)	June 1985	29	1.3	49	0.6	23
	November 1985	16	3.9	38	0.8	26
	June 1986	14	1.4	57	0.8	23
	Mean		2.2	48	0.7	24
Little Econlockhatchee (7.5 km)	June 1985	31	0.1	68	1.3	11
	December 1985	22	0	75	1.5	10
	July 1986	24	0	80	1.2	9
	Mean		0.04	74	1.3	10
Wacissa (16 km)	June 1985	23	11	9	1.3	39
	November 1985	14	7.1	14	1.2	69
	July 1986	25	11	6	1.2	83
	Mean		9.7	10	1.2	64
Wekiva (19 km)	July 1985	34	1.3	34	0.9	50
	November 1985	20	2.6	26	1.0	70
	June 1986	27	1.4	26	0.9	51
	Mean		1.8	29	0.9	57
Alafia (16 km)	July 1985	29	0.2	42	0.9	15
	November 1985	28	0.2	35	0.9	14
	August 1986	26	0	35	1.7	18
	Mean		0.1	37	1.1	16
Reedy Creek (7.5 km)	July 1986	14	0	86	0.8	10
Pottsburg Creek (9.5 km)	June 1986	12	0	33	1.4	28
Mills Creek (14 km)	June 1986	20	0.3	26	2.1	18
St. Marks (10 km)	July 1986	18	3.9	26	1.7	45
Econlockhatchee (11 km)	July 1986	22	0	50	1.0	10
Hillsborough (10 km)	August 1986	22	3.7	35	2.0	31
Hogtown (3.0 km)	June 1986	16	0.3	72	0.2	4
Santa Fe (16 km)	June 1986	16	0.1	39	0.9	14

TABLE 2. Annual TP and TN exports, TP and TN content in plant tissue, and percent of TP and TN exports in plant tissue for Little Wekiva River using measured mean and maximum (September) plant standing crops in 1985.

	Mean 1985 plant standing crop	Maximum 1985 plant standing crop	Growing period May–October 1985
Annual TP export (kg/yr)	39 000	39 000	21 000
TP in plant tissue (kg)[a]	80	94	94
Percent of TP export in plant tissue	0.2	0.2	0.5
Annual TN export (kg/yr)	114 000	114 000	59 000
TN in plant tissue (kg)[a]	500	590	590
Percent of TN export in plant tissue	0.4	0.5	1.0

[a]Assuming 95% water weight for all plants and a phosphorus content of 3.7 mg/g dry weight of plant tissue and a nitrogen content of 23 mg/g dry weight of plant tissue.

(0.3 m^3/s), Palm Spring (0.15 m^3/s), and Starbuck Spring (0.5 m^3/s). These springs and some small groundwater seeps discharge to the Little Wekiva River at the base of the Apopka Hills. TP and TN concentrations averaged greater than 0.1 and 0.3 mg/L, respectively, in Sanlando Spring (TP = 0.17 mg/L, TN = 0.55 mg/L), Palm Spring (TP = 0.13 mg/L, TN = 0.55 mg/L), and Starbuck Spring (TP = 0.15 mg/L, TN = 0.37 mg/L). After the water from the springs had completely mixed with the stream water (0.7 km), in-stream TP averaged 0.36 mg/L and TN averaged 1.1 mg/L along the entire length of the stream. Constant nutrient conditions occurred primarily because the water residence time in the Little Wekiva River averaged 12 h in 1985 and was reduced to less than 2 h when heavy rains occurred.

The above-ground standing crop of aquatic macrophytes (including large algae) in the Little Wekiva River was estimated at 90 transects established along the length of stream in March 1985 and 32 transects in September 1985. Standing crops averaged 1.3 kg fresh weight/m^2 in March and 2.0 kg fresh weight/m^2 in September (Table 1). Standing crop estimates for individual sampling quadrats ranged from 0 to 13 kg fresh weight/m^2, with the greatest abundance of plants occurring in the lower, wider portions of the stream where the forest canopy was open. There was no relationship between the abundance of aquatic macrophytes and nutrient concentrations along the length of the Little Wekiva. To determine if there were sufficient nutrients present in the Little Wekiva River to support the measured standing crops of aquatic macrophytes, we estimated what percentage of the annual TP and TN exports from the stream would be used by the plants, assuming all nutrients had to be extracted from the water (Table 2). Our estimates indicated that <2% of the annual phosphorus and nitrogen exported from the Little Wekiva River would be needed to support the mean or maximum (September, Table 1) measured standing crops of macrophytes in 1985. Even if we restricted our estimates to the May–October period of maximum growth, the plants would need <2% of the nutrients exported from the Little Wekiva (Table 2).

We compared average macrophyte standing crops with nutrient levels in eight additional streams during 1985 to determine if plant abundance was correlated with the level of nutrient enrichment (Table 3). Average macrophyte standing crops in the streams ranged from 0 to 11 kg fresh weight/m^2 (Table 1). The highest above-ground standing crops of aquatic macrophytes were measured in the Wascissa River (11 kg fresh weight/m^2 in June and 7.1 kg fresh weight/m^2 in November), which had an average TP concentration of 0.04 mg/L and an average TN concentration of 0.20 mg/L (Table 3). The other nonpolluted spring-fed streams also supported either equivalent or greater above-ground standing crops of aquatic macrophytes than the Little Wekiva River (Table 1) despite having lower nutrient concentrations (Table 3). The percentage of the annual TP and TN exports that would be needed to support the measured above-ground standing crops was <3% for all streams except Alexander Springs (7.5% for TP and 4.5% for TN) and Wacissa River (12% for TP and 15% for total TN) which had the lowest nutrient concentrations and greatest average above-ground macrophyte standing crops measured in our study (Tables 1 and 3).

In 1986, we surveyed 17 streams to determine if plant abundance was related to the level of nutrient enrichment (Table 4). Mean above-ground standing crops ranged from 0 to 11 kg fresh weight/m^2 (Table 1). Again, some of the highest macrophyte standing crops were measured in nonpolluted spring-fed streams (Alexander Springs, Ichetucknee River, Rock Springs, Wacissa River, St. Marks River, and Hillsborough River) (Table 1). Our estimates of the percentage of the yearly exports of TP and TN contained in the macrophyte beds of the survey streams averaged <2% for all but two streams, Alexander Springs and Wacissa River (Table 4).

Macrophyte abundance in the Little Wekiva River during September 1985 was significantly ($p < 0.05$) correlated with the percent forest canopy coverage ($r = -0.77$), and our estimates of percent forest canopy coverage were significantly correlated with light availability (microeinsteins per square metre per second) reaching the stream surface, as measured by a LiCor model LI-185B quantum radiometer photometer equipped with a LI-190SB quantum sensor ($r = 0.76$). Using mean transect data ($n = 661$) from all streams and all years (Table 5), the average (SC_{avg}) and maximum (SC_{max}) above-ground standing crops of aquatic macrophytes in the Florida streams were inversely related to the percent forest canopy cover (%C) and the relationships can be described by the following logarithmic regression equations:

$$(1)\quad \log (SC_{avg}) = 1.06 - 0.026\ (\%C) \qquad R^2 = 0.93$$
$$(2)\quad \log (SC_{max}) = 1.54 - 0.014\ (\%C) \qquad R^2 = 0.94.$$

Discussion

Nutrient concentrations (especially phosphorus) in Florida streams are naturally high due to edaphic factors (Odum 1953; Omernik 1977). TP and TN concentrations in nonpolluted spring-fed streams typically exceed 0.03 and 0.3 mg/L, respectively, and these streams support an abundance of aquatic macrophytes (Rosenau et al. 1977). Our study demonstrates that aquatic plants in most Florida streams probably need <2% of the annual nutrient exports. This finding agrees with those of Casey (1977) and Madsen (1986) who studied northern streams. Our estimates are also probably highly conservative because many studies have demonstrated that rooted aquatic

TABLE 3. Annual TP and TN exports, TP and TN content in plant tissue, percent TP and TN exports in plant tissue, and mean TP and TN concentrations for all survey streams in 1985.

Stream	Annual TP export (kg/yr)	TP in plant tissue (kg)[a]	Percent of yearly export in plant tissue	Annual TN export (kg/yr)	TN in plant tissue (kg)[a]	Percent of yearly export in plant tissue	Mean TP concentration (mg/L)	Mean TN concentration (mg/L)
Alexander Springs	6 100	460	7.5	62 000	2 800	4.5	0.04	0.41
Ichetucknee Springs	19 000	120	1.0	98 000	710	0.7	0.05	0.26
Alligator Creek	12 000	4.4	<0.1	28 000	28	0.1	0.38	0.82
Rock Springs	6 900	140	2.0	99 000	900	0.9	0.08	1.25
Little Econlockhatchee	34 000	1.5	<0.1	400 000	9.5	<0.1	0.23	2.60
Wacissa	13 000	1 500	12	59 000	9 100	15	0.04	0.20
Wekiva	71 000	410	0.6	330 000	2 600	0.8	0.21	0.88
Alafia	380 000	8.9	<0.1	240 000	55	<0.1	1.95	1.31

[a]Assuming 95% water weight for all plants and a phosphorus content of 3.7 mg/g dry weight of plant tissue and a nitrogen content of 23 mg/g dry weight of plant tissue.

TABLE 4. Annual TP and TN exports, TP and TN content in plant tissue, and percent of TP and TN exports in plant tissue for all survey streams in 1986.

Stream	Annual TP export (kg/yr)	TP in plant tissue (kg)[a]	Percent of yearly export in plant tissue	Annual TN export (kg/yr)	TN in plant tissue (kg)[a]	Percent of yearly export in plant tissue
Little Wekiva	48 000	170	0.4	160 000	1 000	0.6
Alexander Springs	7 400	480	6.5	48 000	3 000	6.3
Ichetucknee Springs	15 000	130	0.9	100 000	830	0.8
Alligator Creek	7 600	3.9	0.1	28 000	24	0.1
Rock Springs	12 000	71	0.6	200 000	440	0.2
Little Econlockhatchee	36 000	<0.1	<0.1	690 000	<0.1	<0.1
Wacissa	15 000	2 700	18	200 000	17 000	8.5
Wekiva	61 000	240	0.4	350 000	1 500	0.4
Alafia	790 000	<0.1	<0.1	500 000	<0.1	<0.1
Reedy Creek	6 800	<0.1	<0.1	490 000	<0.1	<0.1
Pottsburg Creek	170 000	<0.1	<0.1	740 000	<0.1	<0.1
Mills Creek	44 000	14	<0.1	140 000	84	0.1
St. Marks	18 000	320	1.8	220 000	2 000	0.9
Econlockhatchee	27 000	<0.1	<0.1	760 000	<0.1	<0.1
Hillsborough	130 000	210	0.2	450 000	1 300	0.3
Hogtown	15 000	0.7	<0.1	14 000	4	<0.1
Santa Fe	96 000	4.1	<0.1	480 000	26	<0.1

[a]Assuming 95% water weight for all plants and a phosphorus content of 3.7 mg/g dry weight of plant tissue and a nitrogen content of 23 mg/g dry weight of plant tissue.

macrophytes can obtain a large fraction of their nutrients from the hydrosoil (Bristowe and Whitcombe 1971; Carignan and Kalff 1980). We therefore conclude that nutrients are not the factor limiting the abundance of aquatic macrophytes in Florida streams and that the control of nutrients entering Florida streams from wastewater treatment plants will not reduce the aquatic weed problems even though nutrient concentrations could be reduced significantly.

Butcher (1933) observed that the chief factor governing the distribution and abundance of aquatic macrophytes in English streams was water current. Other factors that he recognized as important included bottom substrate and light availability. Average current velocity in our Florida streams was low (<36 cm/s) and very few sections of the streams had sufficient current to limit the abundance of aquatic macrophytes. Because current velocity was low, bottom substrates in most of the study streams were dominated by sand, silt, and silty clays, which provide excellent substrate for the growth of aquatic macrophytes (Butcher 1933). Only the Alafia River and portions of Ichetucknee River had unsuitable bottom substrates (limestone outcrops) for macrophytes.

Our study suggests that the chief factor limiting the growth of aquatic macrophytes in Florida streams is light availability, although substrate type, water depth, and current velocity were

TABLE 5. Mean, minimum, and maximum plant standing crop for consecutive 10% canopy cover groups. Data are from 17 Florida streams sampled during 1985 or 1986.

Percent canopy	Number of transects	Plant standing crop (kg fresh wt/m²)		
		Mean	Minimum	Maximum
0.0–9.9	134	6.0	0	35
10.0–19.9	59	5.3	0	20
20.0–29.9	84	2.5	0	16
30.0–39.9	75	1.4	0	8.9
40.0–49.9	52	1.0	0	10
50.0–59.9	54	0.3	0	5.0
60.0–69.9	37	0.5	0	4.0
70.0–79.9	28	0.2	0	3.1
80.0–89.9	20	0.2	0	5.1
90.0–100	118	0.01	0	1.0

limiting macrophyte standing crops in some sections of our streams. During the aquatic macrophyte surveys, it was visually obvious that the distribution and abundance of aquatic macrophytes were governed primarily by openings in the forest canopy coverage. Equations 1 and 2 demonstrate strong relationships between forest canopy coverage and potential mean and maximum above-ground standing crops of aquatic macrophytes. This is similar to the findings of Dawson and Kern-Hansen (1978, 1979) who demonstrated a strong inverse relationship between light available at the stream surface and the biomass of aquatic macrophytes in English and Danish streams. Our data, however, indicate that macrophyte standing crops increase rapidly only after the forest canopy coverage is reduced below 50%, but macrophyte standing crops in streams with limited or no forest canopy coverage can be limited by light availability when water depths exceed the depth of light penetration. The control of aquatic weed problems in Florida's small (<50 m width), shallow (<2 m), slow-flowing, nutrient-rich streams and other similar streams, therefore, depends in part on the maintenance of a forest canopy cover.

Assessing macrophyte standing crops and nutrient loads in streams is difficult, but our study and others (e.g. Kern-Hansen and Dawson 1978) suggest that nutrient control through the diversion of wastewaters offers no solution for aquatic weed problems in flowing water systems with background TP concentrations exceeding 0.03 mg/L. In small, nutrient-rich streams, maintenance of the forest canopy cover and reestablishment of the canopy cover where it has been removed are probably the only feasible natural methods of controlling aquatic weed problems. In larger streams or stream reaches where the forest canopy is open naturally, nuisance growths of aquatic macrophytes will have to be controlled through management techniques such as mechanical harvesting (Westlake and Dawson 1982) or use of aquatic herbicides. Nutrient control might prove useful for the control of aquatic macrophytes in oligotrophic streams (TP concentrations <0.01 mg/L), but additional research will be needed to establish critical nutrient concentrations.

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An Evaluation of Lake Trout (*Salvelinus namaycush*) Hooking Mortality in the Upper Great Lakes[1]

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Investigation of lake trout (*Salvelinus namaycush*) hooking mortality was conducted in Lakes Michigan, Huron, and Superior during 1984 and 1985. Fish were caught and handled by charterboat operators and sport fishermen. Mortality was estimated by returning hooked fish to the lake after tethering them to a line–buoy system. Overall hooking mortality was estimated to be 14.9% (95% C.I. = 7.4–25.7). Significantly higher mortalities occurred in fish that were not discovered to have been hooked and in fish of smaller size classes. In addition, lake trout that were hooked in internal regions produced a mortality of 71.4% whereas those hooked in the upper or lower jaw had a mortality of 6.9%. The depth from which the fish was angled, the temperature differential from this depth to the surface, gear type, and handling times had no significant impact on survival. These results support the use of size limits, creel limits, and season restrictions as effective methods to reduce sport fishing mortality of lake trout.

En 1984 et 1985, on a étudié la mortalité du touladi (*Salvelinus namaycush*) attribuable à la prise par hameçon dans les lacs Michigan, Huron et Supérieur. Les poissons ont été capturés et manipulés par des exploitants de bateaux nolisés et des pêcheurs sportifs. On a estimé la mortalité en retournant les poissons pris à l'hameçon dans le lac après les avoir attachés à un dispositif de lignes et bouées. On a estimé à 14,9 % (I.C. à 95 % = 7,4–25,7) la mortalité globale attribuable à la prise par hameçon. La mortalité a été significativement plus élevée chez les poissons qui ne semblaient pas avoir été pris à l'hameçon et chez les poissons des classes de tailles plus petites. En outre, la mortalité chez les touladis où l'hameçon s'était fixé dans les régions internes a été de 71,4 % alors qu'elle a été de 6,9 % chez ceux où l'hameçon s'était fixé à la mâchoire supérieure ou inférieure. La profondeur à laquelle les poissons ont été capturés, l'écart de la température entre cette profondeur et la surface, le type d'engin, et les durées de manipulation n'ont pas eu de répercussions significatives sur la survie. Ces résultats viennent appuyer l'utilisation de limites de taille, de limites de prises et de restrictions par saison de pêche comme méthodes efficaces permettant de réduire la mortalité du touladi par pêche sportive.

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Lake trout (*Salvelinus namaycush*) were once abundant in the Great Lakes and constituted one of the major predators in this system (Christie 1974). Until the 1950's, naturally reproducing stocks of this fish helped to support a thriving commercial fishery in the Great Lakes. Historically, Lakes Michigan and Huron each consistently produced in excess of 500 000 lb (227 000 kg) of lake trout annually, while the harvest in Michigan waters of Lake Superior reached over 400 000 lb (181 600 kg) per year (Baldwin et al. 1979). Intensive overfishing, environmental degradation, and the invasion of the sea lamprey (*Petromyzon marinus*) through the Welland Canal have often been cited as the causes of the total collapse of the lake trout stocks in Lakes Michigan and Huron and the near collapse of the stocks in Lake Superior (Christie 1974).

With the development of a chemical lampricide (trifluoromethylnitrophenol) and subsequent reductions of the sea lamprey populations, along with extensive limitations on commercial fishing, management personnel felt that a suitable environment had been created for the reestablishment of lake trout stocks (Peck 1984). Stocking of fingerlings in Lake Michigan began in 1965 with the intention of establishing self-sustaining stocks of this fish. However, to date, only Lake Superior shows signs of significant natural recruitment (Rybicki 1983).

Many theories have been advanced regarding the failure of reproduction in the planted lake trout stocks. Degradation of spawning habitat through changing land use practices, increased contaminants in the Great Lakes, lack of abundant spawners, genetic inviability in introduced strains, and failure of lake trout to locate and deposit eggs successfully on suitable reefs have all been cited as possible reasons for this failure (Rybicki and Keller 1978; Peck 1979). Attention has recently shifted to achieving the necessary critical biomass of reproductive age fish in the population.

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To achieve this necessary critical mass, the Great Lakes Fishery Commission Lake Michigan Lake Trout Technical Committee (Eshenroder et al. 1984) advised that total annual mortality not exceed 40%. Currently, mortality exceeds this in most statistical management districts of Lake Michigan (Rybicki 1983). Records available from the Michigan Department of Natural Resources indicate that the majority of lake trout do not reach maturity until the age of 6 yr in Lake Michigan. As a result, the biomass of fish reaching maturity constitutes a relatively small percentage of the total biomass of the stocks.

In an attempt to increase spawner abundance, the Michigan Department of Natural Resources established creel limits and season restrictions aimed at reducing the mortality of adult fish. The ultimate success of these regulations depends upon the degree of mortality experienced by lake trout that have been caught and released by anglers, but an estimate of this hooking mortality has not been available. Controversies arose among managers and between managers and anglers as to the effectiveness of regulations in reducing lake trout mortality. Prior hooking mortality studies on salmonid species indicated highly variable results ranging from 3.3% for Atlantic salmon (*Salmo salar*) (Warner 1976) to 90% for rainbow trout (*Salmo gairdneri*) (Mason and Hunt 1967). Hooking mortality of lake trout was reported to be 6.98% in Great Slave Lake, Canada (Falk et al. 1974).

The purpose of this study was to evaluate the degree of hooking mortality of lake trout in Lakes Michigan, Huron, and Superior. Relationships between mortality and various physical and biological factors were investigated.

Methods

To collect data, research personnel accompanied Great Lakes charter boat operators and sport fishermen on fishing trips. In addition, the use of a research vessel augmented fishing time in 1985.

Three of the upper Great Lakes were sampled in 1984 and two in 1985. Eight ports were visited along the eastern shore of Lake Michigan and one port each on Lakes Huron and Superior in 1984 only (Fig. 1). In 1985, Lake Superior was excluded due to inclement weather at the scheduled time of sampling. Different ports were visited to obtain samples from different fish stocks and also to provide a cross-section of Great Lakes fishing techniques.

Lake trout were caught by both fishermen and research personnel using traditional Great Lakes fishing methods. Most fish were caught by trolling artificial lures attached to downriggers. However, in five instances, alternate methods such as wire line fishing were used by fishermen. At the time that the fish was brought on board, research personnel recorded information on depth of capture, water temperature at the depth of capture, anatomical site of hooking, lure type, time required to land the fish (playing time), time the fish was onboard (deck time), and surface water temperature.

FIG. 1. Sampling stations for lake trout hooking mortality in 1984 and 1985.

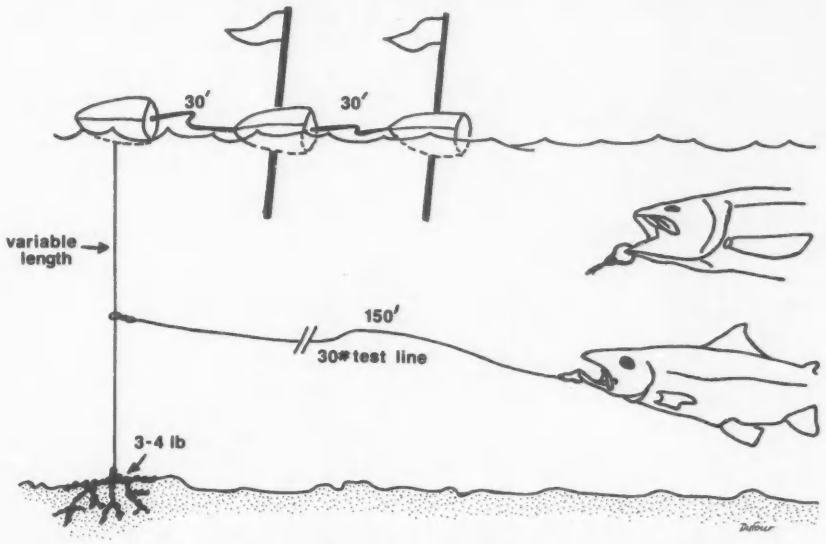

FIG. 2. Schematic diagram of the line–buoy method used to assess lake trout hooking mortality.

TABLE 1. Hooking mortality of lake trout returned to natural waters in the Upper Great Lakes, 1984–85. Confidence intervals from Mainland et al. (1956).

Year	Number caught	Number of mortalities	Percent mortality	Confidence intervals (95%)
1984	22	3	13.6	3.2–37.9
1985	45	7	15.6	6.5–29.5
Combined	67	10	14.9	7.4–25.7

To assess mortality, fish were tethered to a buoy and line system and returned to the water. This involved placing a metal clip through the center of the lower mandible (Fig. 2). The clip was connected via a two-way swivel to 45–70 m of monofilament line (15- or 30-lb test) which was in turn attached to a crab pot buoy. The fish, line, and buoy were returned to the water where the lake trout could seek out its preferred temperature and depth stratum. Horizontal movement was restricted to the length of the tether (45–70 m). In 1984, the buoys were unanchored and fish were left out for 1–7 h (dependent upon the duration of the fishing trip). In 1985, an anchor was attached to the buoy, but the fish were still able to move to their preferred depth (Fig. 2). Ideally, in 1985, fish were retained on the buoy system for a minimum of 48 h, since 90–95% of hooking mortality in salmonids had been shown to occur within 48 h of capture (Mongillo 1984). However, fishermen who came too close to this system often became entangled in the line. Therefore, release times were shortened during times of heavy fishing pressure.

After the lake trout were retrieved, they were classified as alive or dead. Total length, weight, sex, and evidence of lamprey scars were recorded for each fish. Age was determined with Michigan Department of Natural Resources fin clip records and verified with scale aging techniques.

Statistical Analysis

All factors or classifications of factors were compared to determine their impact on observed mortality. Chi-square contingency table analysis was used in all cases. Some data were categorized into groups for analysis. In these cases, the group intervals were the smallest that incorporated a sufficient number of observations in each cell that allowed for accurate testing using chi-square. In all comparisons, a 0.95 significance level was used.

Although the effects of interactions between the variables were of interest, the small sample size precluded the use of multiple comparison tests. Several attempts were made to fit the variables into a log-linear model, but again the nature of the data and the small sample size invalidated this approach.

TABLE 2. Mortality by length of lake trout caught in the upper Great Lakes, 1984–85.

Total length (mm)	Number caught	Number of mortalities
457–508	7	4
508–559	8	0
559–610	13	1
610–660	14	1
660–711	15	2
711–762	6	1
762–813	1	0

Results and Discussion

Over the two years of sampling, a total of 67 lake trout were returned to the water on the line–buoy system. In 1984, 13.6% of the lake trout caught and released using the buoy system died. In 1985, 15.6% died (Table 1). There was no significant difference in mortality between the two years (χ^2 (1 df) = 0.0429, $p > 0.05$). For the 2-yr period, overall hooking mortality was 14.9% (Table 1). Although the sample size was small, the agreement of the estimates for the two years provides strong support for the overall mortality estimate. This rate is higher than that found by Falk et al. (1974) who determined that 6.98% of the caught and released lake trout died. However,

several differences exist between Falk's work and this study. The work done by Falk was carried out in Great Slave Lake, Canada, where fish were taken under different conditions and using different angling methods. Also, Falk used floating live cages to evaluate hooking mortality. The difference in the estimates between the two studies could be a result of different methodologies. In addition, severe temperature changes as experienced by fish caught from the Great Lakes did not occur in Falk's study.

Size

Fish ranged in size from 461 to 801 mm, with the average being 617 mm. Mortalities were noted over the entire size range. When fish lengths were placed into 51-mm (2-in.) size categories (Table 2), there was found to be a significant difference in mortality between various size classes (χ^2 (6 df) = 7.359, $p < 0.05$). The smallest size category of fish experienced the greatest mortality. Contrary to these results, Wydoski et al. (1976) concluded that larger rainbow trout, when caught and released, were stressed to a greater degree than smaller ones under the same conditions. However, Pelzman (1978) found no correlation between the size of largemouth bass (*Micropterus salmonides*) and mortality.

Handling Times

The playing time, or the approximate time elapsed from the moment of initial hooking and the moment of landing the fish, ranged from 53 s to 5 min and 3s. Times were grouped into 1-min intervals, and mortality did not differ among the times (χ^2 (3 df) = 0.7440, $p > 0.05$). Marnell and Hunsaker (1970), working with cutthroat trout (*Salmo clarki*), reported that mortality related to playing times of up to 10 min were not significantly different from their control group. However, Bouck and Ball (1966) found that 87% of rainbow trout that were angled to exhaustion died. Due to the relatively short playing times of lake trout in this study, it is doubtful that many were physically exhausted when landed.

Several fish were not immediately discovered after they were hooked. These fish were omitted from the analysis of playing times (but not overall mortality estimates), since accurate times could not be established. When the mortality estimates of these fish were compared with the estimates of other angled fish, a significant difference in mortality was observed (χ^2 (1 df) = 6.3849, $p < 0.05$). The fish that were not discovered immediately upon hooking produced a mortality estimate of 50% compared with 11.4% mortality for all other fish. Fish fighting the drag of the boat may accumulate a lethal level of the by-products of anaerobic muscular activity (i.e. lactic acid). There may be a reduced ability to pass water over the gills resulting in suffocation. Joans and Randall (1978) have discussed mechanisms by which this could happen. Thus, these fish may be stressed from oxygen deficiency by the time that they are brought onboard and the results would therefore coincide with those reported by Bouck and Ball (1966).

The amount of time that the fish spent out of the water (deck time) did not significantly influence the survival of hooked lake trout (χ^2 (3 df) = 5.508, $p > 0.05$). Deck times ranged from 1 min and 10 s to 4 min and 30 s and were categorized into 1-min intervals for analysis. This was apparently not long enough to cause severe physiological damage to the fish.

Gear Type

There were three main lure types used by anglers. These consisted of small plug-like lures with small treble hooks (known

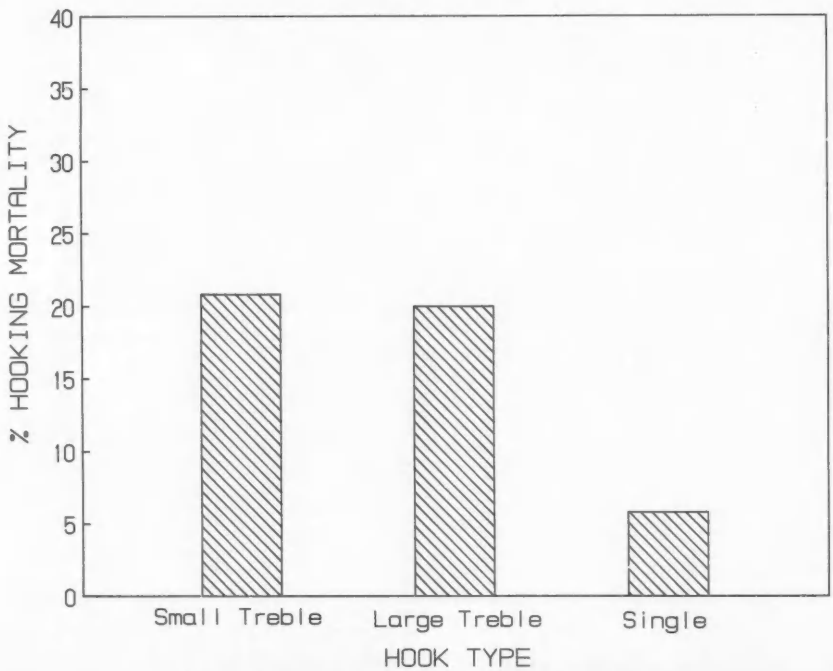

FIG. 3. Hooking mortality associated with different hook types.

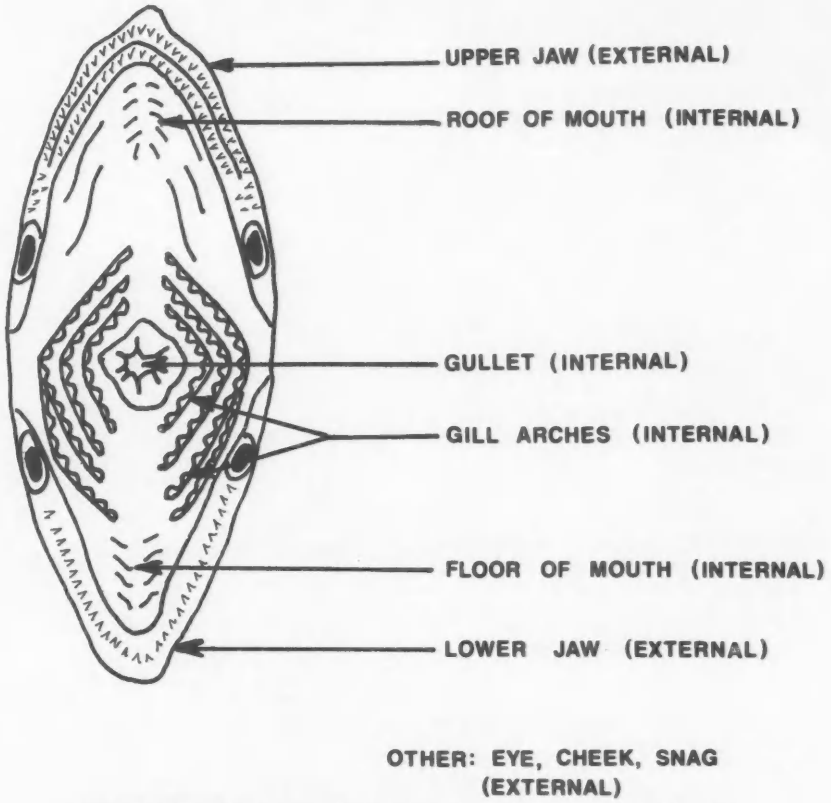

FIG. 4. Classification of anatomical hooking sites (modified from Mongillo 1984).

TABLE 3. Number of lake trout caught at individual water temperature differentials in the upper Great Lakes, 1984–85. Numbers in parentheses indicate the number of mortalities.

Water temperature at depth (°C)	Surface water temperature (°C)																	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
4	1												2	1	4			
5										2								
6					3			4					2(1)	1		1		
7			5(4)		1		1								1			
8		1			2										1			1
9										1		4		1	2		2(1)	
10															2			
11					1										2			
12							1				2	1						1(1)
13	1										1(1)							
14											1							2(1)
20															1			

as p-nuts), spoons with large treble hooks, and spoons with large single hooks. Although higher mortalities were observed with p-nuts and large treble hook spoons (Fig. 3), these differences were not found to be significant (χ^2 (2 df) = 1.905, $p > 0.05$). These findings agree with those of Falk et al. (1974) and Dotson (1982). However, in a summary of hooking mortalities of salmonids, Mongillo (1984) concluded that the practice of using single hooks on lures causes slightly higher mortalities than treble hooks.

Anatomical Hooking Site

For statistical analysis, hooking sites were classified as being internal or external (Fig. 4). Approximately 72% of the lake trout in this study were hooked in the upper or lower jaw (external). These fish experienced a mortality of 6.9%, while fish that were hooked in one or more internal sites exhibited a mortality of 71.4%. A significant difference therefore exists in mortality between the two classifications (χ^2 (1 df) = 21.805, $p < 0.05$). This difference is a function of damage to internal struc-

tures and subsequent stress of the fish. Other investigators have similarly found hooking site to be important in determining mortality of hooked fish. Falk et al. (1974) concluded that the primary cause of death in hooked lake trout was attributable to hook placement and bleeding. Hook placement in the gill arches produced excessive bleeding and increased mortality. Pelzman (1978) found that significant mortality occurred among largemouth bass that had been hooked in the esophageal region. In addition, Warner (1979) indicated that hooking mortality of landlocked Atlantic salmon was more a function of hooking site (swallowing the hook) than gear type.

Temperature

The difference between the temperature at the depth at which the lake trout was hooked and the surface water temperature did not appear to have an effect on mortality (Table 3). When fish observations were placed into categories of temperature differential (0–5, 6–10, or 11–15°C), no significant differences were observed between the three categories (χ^2 (2 df) = 1.347, $p > 0.05$). This conclusion is consistent with that of Marnell and Hunsaker (1970) who found that the survival of cutthroat trout after hooking and releasing was not affected by surface water temperatures between 37 and 62°F (2 and 17°C). However, relationships have been established which suggest an increasing mortality with increasing water temperatures (Benson and Bulkley 1963; Klein 1965; Dotson 1982).

Depth

Lake trout were caught from depths ranging from 7.56 to 49 m. No significant difference was observed among depth ranges in the probability of death (χ^2 (4 df) = 4.327, $p > 0.05$). When fish are raised from great depths, a decrease in pressure at the rate of 1 atmosphere (101.325 kPa) for every 10 m allows gas in the gas bladder to expand. Since lake trout are physostomous, they possess the capability of releasing pressure from their expanding gas bladder via the esophageal connection. Often, when lake trout were brought to the surface, they could be heard releasing gas from the gas bladder. Several fish that ultimately survived were observed floating on the surface for periods of up to 10 min, possibly as a result of the extended gas bladder.

Management Implications

The primary objective of this study was to determine the degree of hooking mortality experienced by lake trout that are caught and released by Great Lakes anglers. This was in an effort to determine the effect of various fishing regulations upon the overall mortality of lake trout stocks and the ultimate plans for achieving their natural reproduction in the Great Lakes. Currently, two types of restrictions are imposed on lake trout fishermen. A creel limit of two lake trout per angler per day is imposed on fishermen in Lake Michigan, while the limit is three in Lakes Superior and Huron. Based on the results of this study, the relatively low overall mortality appears to support the use of this limit as a method of reducing sport fishing mortality. Great Lakes fishermen are often able to target for alternate species (such as salmon), and therefore the incidental catch of lake trout after the daily limit has been reached may not be significant. However, lake trout that are incidental to the catch will exhibit a high survival when released, according to these results.

The recently imposed season limit by the Michigan Department of Natural Resources may also be effective in reducing fishing mortality. Although the current season closes on August 15 when surface water temperatures are often high, we found no evidence which indicated an increasing mortality with increasing differential in water temperatures. Lake trout still have a substantial chance for survival in the warmer surface waters. The basis for establishing opening and closing dates for lake trout fishing therefore would depend more upon the evaluation of fishing pressure during different times of the year and socioeconomic considerations. Tourism connected with Great Lakes fishing contributes substantially to the economy of Michigan. Many businesses would prefer to extend the lake trout season to Labor day, the traditional end of the tourist season.

Imposing a size limit for lake trout has also been under consideration by management agencies. As was noted earlier, mortalities occurred over the entire size range of observed fish, but the smallest size classes of fish showed the highest mortality. Generally, significant maturity in lake trout is not reached until the age of 6 yr, at which time the fish are approximately 660 mm in length (Rybicki and Keller 1978). If this was imposed as the minimum size for lake trout harvest, approximately 66% (42/64) of the fish caught in this study would have been returned to the water. Of these returned fish, 14.3% would have died. It should be noted that this estimate is actually below the estimate for overall mortality. This is because the sample size for calculations on length was reduced to 64 fish due to missing length data caused by gull predation in one case and loss of live fish upon retrieval in two cases. Thus, a size limit designed to allow a greater number of fish to reach maturity may actually be an effective method to increase spawner biomass.

Clark and Huang (1985) provided a lake trout population model which incorporates varying fishing levels and mortality rates. With this model, they simulated population changes in one statistical management district of Lake Michigan (Frankfort – Good Harbor Bay, statistical district MM5). Given that stocking remains at historical levels and that mortality and maturity schedules are accurate (Table 4), the model predicts that rehabilitation will not be achieved in 25 yr, as was noted by Clark and Huang (1985). Rehabilitation was defined by the authors as the natural production of 25 000 4-yr-olds, the

TABLE 4. Parameters incorporated into lake trout population model of Clark and Huang (1985).

Age (yr)	Number in original population	Natural mortality	Percent mature
1	0	0.46	0
2	63 128	0.46	0
3	39 851	0.46	0
4	25 157	0.46	6
5	13 711	0.36	17
6	7 677	0.36	92
7	4 298	0.36	97
8	2 406	0.36	100
9	1 576	0.36	100
10	762	0.36	100
11	439	0.36	100
12	215	0.36	100
13	147	0.36	100
14	63	0.36	100
15	44	0.36	100

Size of vulnerability to gear = 540 mm
Instantaneous fishing mortality = 0.22
First year survival = 0.005
Eggs per kilogram per female = 1850

approximate number now achieved by stocking. After 25 yr, with the size limit of 660 mm and the hooking mortality rate of 14.9%, approximately 13 000 4-yr-olds will be produced.

Conclusion

The objective of this study was to estimate the mortality that is experienced by lake trout that are caught and released by anglers in the Great Lakes. Overall hooking mortality was found to be 14.9%. Fish hooked in one or more internal areas experienced significantly higher mortality (71.4%) than those hooked in the upper or lower jaw (6.9%). In addition, fish that were not immediately discovered upon hooking exhibited a mortality of 50.0%, while those that were immediately landed exhibited 11.4% mortality, creating a significant difference between the two classifications. The smallest size classes of fish died at a slightly higher rate than larger ones, although this mortality is still relatively low. These conclusions support the use of season limits, size restrictions, and creel limits as a means of reducing sport fishing mortality of lake trout stocks in the Great Lakes. However, even with these restrictions, the rehabilitation of lake trout stocks in the Great Lakes may not be readily achieved.

Acknowledgments

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Comparison of Phosphorus Dynamics in Two Oklahoma Reservoirs and a Natural Lake Varying in Abiogenic Turbidity[1]

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Heath, R. T., and D. A. Francko. 1988. Comparison of phosphorus dynamics in two Oklahoma reservoirs and a natural lake varying in abiogenic turbidity. Can. J. Fish. Aquat. Sci. 45: 1480–1486.

The rate of phosphate uptake, the immediate fate of phosphate, and the potential rate of release of phosphate from dissolved phosphorus compounds in epilimnia of two reservoirs and one natural lake in northcentral Oklahoma were compared in the summer and in the late autumn. Sangre Isle Lake is a highly productive eutrophic impoundment, Sanborn Lake is a natural lake with low phytoplanktonic productivity, and Boomer Lake is an impoundment with modest productivity that is heavily affected by clay suspensoids. Although the uptake of phosphate (radiometrically determined) was rapid in each of these lakes in summer, the fate of phosphate in Boomer Lake differed from that of the others, with much of the phosphate sorbing to suspended silts and clays rather than biota (determined by isopycnic sedimentation). Recycling of phosphate from phosphomonoesters was greatest in Sangre Isle Lake, moderate in Sanborn Lake, but was undetectable in Boomer Lake. These findings suggest that high concentrations of clay suspensoids influence the epilimnetic P dynamics in reservoirs and lakes.

Le taux d'absorption du phosphate, le devenir immédiat du phosphate et le taux potentiel de libération de phosphate à partir de composés de phosphore dissous dans l'épilimnion de deux réservoirs et d'un lac naturel dans le centre-nord de l'Oklahoma ont été comparés en été et à la fin de l'automne. Le lac Sangre Isle est un réservoir eutrophe très productif, le lac Sanborn est un lac naturel où la productivité phytoplanctonique est faible, et le lac Boomer est un réservoir ayant une productivité modeste qui dépend beaucoup des suspensoïdes d'argile. Bien que l'absorption du phosphate (déterminée par radiomètre) était rapide dans chacun de ces lacs en été, le devenir du phosphate dans le lac Boomer différait de celui des autres, une bonne partie se liant aux vases et argiles en suspension plutôt qu'au biote (déterminée par sédimentation isopycnique). Le recyclage du phosphate à partir des phosphomonoesters était le plus élevé dans le lac Sangre Isle, modéré dans le lac Sanborn, mais non décelable dans le lac Boomer. Ces résultats donnent à penser que les concentrations élevées de suspensoïdes d'argile influent sur la dynamique du P dans l'épilimnion des réservoirs et des lacs.

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Siltation is a considerable problem, especially in artificial impoundments heavily impacted by runoff from agricultural watersheds. Allochthonous input of clay suspensoids and resuspension of clays within these basins reduce light penetration, thereby directly influencing phytoplankton productivity. Suspensoids in a turbid lake may also affect productivity by influencing the availability of phosphorus either by acting as a source of available P (Yousef et al. 1980) or by sorbing dissolved phosphate (Stumm and Morgan 1981), rendering it unavailable for planktonic growth (Nyffeler et al. 1986). Also, we have previously shown that phosphate and dissolved organic phosphorus (DOP) compounds can sorb differentially to the surfaces of suspended solids, differently affecting the availability of each (Francko and Heath 1981). Therefore, there are reasons to believe that the fate of phosphate and the relative significance of DOP to phytoplankton productivity may be greatly affected by abiogenic turbidity.

This study investigated the influence of abiogenic turbidity on the P dynamics of the planktonic community in an artificial impoundment by comparing the P dynamics in Boomer Lake (heavily silted with low planktonic productivity) with the P dynamics in Sangre Isle Lake (low siltation but high planktonic productivity resulting in turbidity similar to that found in Boomer Lake) and with the P dynamics of Sanborn Lake (low siltation but low planktonic productivity similar to that of Boomer Lake) during the growing season. We compared the rate of phosphate uptake by seston and the immediate fate of phosphate to provide an indication of the relative significance of phosphate uptake by suspended sediments and by biota. The potential rate of release of phosphate from dissolved *P* compounds was used to estimate the importance of P cycling from DOP in each community.

[1] This paper is partly based on a presentation at the P-Plankton Dynamics Symposium during the 27th Conference on Great Lakes Research, International Association for Great Lakes Research, Brock University, St. Catharines, Ont., Canada, May 1984.

Materials and Methods

All samples were collected from the 0.1-m stratum into acid-rinsed polyethylene sterilized bottles, and all procedures were done in acid-rinsed sterilized glassware at ambient temperature and pH, unless otherwise noted. Temperature, conductivity, nephalometric turbidity, alkalinity, and pH were determined by standard limnological methods (Wetzel and Likens 1979). Phytoplankton were observed immediately after collection and without fixation. Chlorophyll *a* was determined fluorimetrically following collection of particulate material on Millipore HA (0.45-μm) filters and extraction of the pigment into alkaline methanol (Wetzel and Likens 1979). Soluble reactive phosphorus (SRP) was determined on water filtered under a vacuum pressure of 0.5 Pa through Millipore HA filters that had been prerinsed in 0.1 M HCl.

Determination of the Rate of Phosphate Uptake

The rate at which a proportion of available phosphate moved from solution to particles was determined radiometrically (Heath 1986). Carrier-free phosphate free of acid (New England Nuclear) was added to freshly collected samples of lake water to give a final activity of approximately 800 Bq·mL^{-1}. One-millilitre aliquots were removed at timed intervals and filtered through Millipore HA (0.45-μm pore size) filters which were presoaked in a 0.5 mol·L^{-1} solution of KH_2PO_4 and postrinsed with distilled deionized water to remove nonparticulate phosphate. Addition of similar amounts of radiolabel to prefiltered water routinely showed that less than 1 Bq was retained on the filter as nonparticulate background.

The filters were dried and counted by liquid scintillation. The apparent first-order uptake rate constant, k, was determined as the slope of the line $\ln [P_0/(P_0 - x_t)]$ versus time, where P_0 was the total activity of 1 mL of lake water and x_t was the activity retained by the Millipore filter at time t (Heath 1986). Other filters used in this study were selected for their different pore sizes: 0.2 μm (Nucleopore), 0.6 μm (Nucleopore), 1.5 μm (Whatman), and 3.0 μm (Nucleopore).

Phosphate uptake exhibited apparent first-order kinetics, permitting estimation of the velocity of phosphate uptake (v) from the first-order rate equation

$$v = k(P)$$

where P is the amount of phosphate available to particles.

We estimated the available phosphate as SRP (Murphy and Riley 1962). Rigler (1966) argued cogently that the results of a radiometric bioassay indicated that SRP in concentrations less than 5 μg·L^{-1} often greatly overestimated available phosphate. He reasoned that other compounds (e.g. dissolved organic compounds) may hydrolyse under the extreme pH used to determine SRP. Our previous attempts to identify such acid-labile compounds in these and other lakes showed that about 90% of the SRP had the same apparent weight-average molecular weight (using gel permeation) and affinity for an anion-exchange resin as phosphate. These observations are consistent with the view that most SRP is phosphate (Francko and Heath 1979; Francko 1984b). Also, Nürnberg and Peters (1984) recently showed with a refined bioassay technique that under most conditions, SRP accurately estimated P availability.

Determination of Bouyant Density of Seston

Clay particles, having bouyant densities greater than 2.0 g·mL^{-1}, can easily be isolated from algal and bacterial cells, which typically have bouyant densities less than 1.15 g·mL^{-1} by using isopycnic sedimentation techniques (Price et al. 1978).

We used the discontinuous gradient method of Wollum and Miller (1980) because most cells could be collected at their isopycnic positions at the interface between two sucrose solutions, while clays and silts migrate to the bottom of the centrifuge tube under relatively gentle sedimentation forces. A 10-mL portion of a 55% sucrose solution (w/v) (bouyant density = 1.30 g·mL^{-1}) was placed at the bottom of 30-mL capacity centrifuge tubes. On top of this was placed a 10-mL portion of a 5% sucrose solution (w/v) (bouyant density = 1.03 g·mL^{-1}). A 2-mL aliquot of radiolabeled lake water was carefully layered on top of the second cushion. The tubes were centrifuged at 3000 × g for 20 min in a Sorvall swinging bucket rotor (model No. HB-4). One-millilitre portions were collected from the top with an automatic pipet, placed into an aqueous liquid scintillation cocktail (Budget-Solve, RPI Corp.), and counted by liquid scintillation.

Release of Phosphate from Phosphomonoesters (PME)

The rate of phosphate release from PME was estimated from the Michaelis–Menten kinetics equation, using values of phosphatase activity and substrate concentration observed in the samples of freshly collected lake water. Phosphatase activity was observed using the fluorimetric technique of Perry (1972), using *o*-methylfluoroscein phosphate as substrate. The "potential rate of recycling" was taken as the V_{max} of the enzyme activity determined on freshly collected lake water. PME concentration was determined as the difference in detectable SRP following exhaustive hydrolysis with calf intestinal mucosa alkaline phosphatase (Sigma Chem Co.) by a procedure we have described previously (Francko and Heath 1979). We assumed a K_m of 1 × 10^{-4} mol·L^{-1}, a typical value for alkaline phosphatases (Fernley 1971) that we have observed elsewhere in freshwater systems (Boavida and Heath 1984; Heath 1985, 1987) and which is a value intermediate among the K_m values observed by Berman (1970): 0.3 × 10^{-4} to 6.7 × 10^{-4} mol·L^{-1}.

Description of Lakes

Each freshwater system is located within Stillwater, Payne County, northcentral Oklahoma (36°N, 97°W). The lakes are generally dimictic, but monomixis can occur in Boomer Lake and Sangre Isle Lake during mild winters.

Sanborn Lake is a small (5-ha) oligotrophic natural lake located in a small wooded watershed with a mean depth of about 2 m. Productivity is dominated by submersed and floating-leaved aquatic vascular plants rather than phytoplankton. Abiogenic turbidity is very low in Sanborn Lake, which generally has a Secchi depth greater than 2 m.

Sangre Isle Lake is a small (3.2-ha) shallow (mean depth of 2.0 m) eutrophic impoundment in a wooded residential watershed. It is dominated by a very productive phytoplankton community in the summer and fall, often having a Secchi depth of less than 0.5 m; it is not heavily affected by clay suspensoids.

Boomer Lake is a mesotrophic reservoir (102-ha surface area) dominated by clay suspensoids, having a Secchi depth less than 0.5 m throughout the year.

Limnological data for the study periods (Table 1) are supplemented in Craven and Brown (1968) and Francko (1983a, 1983b, 1986, 1987). Characteristics of the P metabolism of these communities presented here and elsewhere (Francko

TABLE 1. Limnological characteristics of lakes when sampled. Temperature and pH were determined in situ. Alkalinity, conductivity, nephalometric turbidity, and total chlorophyll *a* were determined in the laboratory shortly after collection. The algal community was examined by light microscopy without fixation and characterized by the dominant phytoplankton.

Date/lake	Temp. (°C)	Alk. (meq $CaCO_3$)	Cond. (μS)	pH	Turb. (N.T.U.)	Chl *a* ($\mu g \cdot L^{-1}$)	Algal community and dominant phytoplankton
17 November 1983							
Boomer	11.7	2.00	340	6.60	67	2.4	Large centric diatoms
Sangre Is.	11.0	1.54	230	6.99	41	13.4	Euglenophytes and Xanthophytes
Sanborn	11.4	1.86	270	6.80	6	0.5	Microflagellates
25 July 1984							
Boomer	30.0	2.16	295	8.12	70	6.4	Microflagellates
Sangre Is.	30.0	1.96	180	8.37	89	168.1	*Anabaena circinalis*
Sanborn	30.0	1.52	220	7.94	11	4.3	*Schroederia* and *Oscillatoria*

TABLE 2. Phosphate uptake rate by seston and isopycnic sedimentation.

Date/lake	SRP ($nmol \cdot L^{-1}$)	Phosphate turnover time (min)	Phosphate uptake *k* (min^{-1})	Phosphate uptake velocity ($nmol \cdot L^{-1} \cdot min^{-1}$)	Percent ^{32}P with a density less than 1.3 $g \cdot mL^{-1}$
17 November 1983					
Boomer	152	301.3	0.003	0.46	62.44
Sangre Is.	16	5.8	0.172	2.75	82.03
Sanborn	Undetectable	19.3	0.052	—	96.20
25 July 1984					
Boomer	271	8.66	0.116	31.4	65.58
Sangre Is.	181	2.6	0.373	67.5	91.39
Sanborn	181	7.8	0.129	23.3	80.4

1983a, 1984a, 1984b, 1986) support the view that P is a limiting nutrient in each of these freshwater systems. Summer epilimnetic SRP concentrations are generally less than 5 $\mu g \cdot L^{-1}$. Epilimnetic alkaline phosphatase activity in Sangre Isle Lake and Sanborn Lake often exceeds 0.5 $nmol \cdot L^{-1} \cdot min^{-1}$ during the summer and fall growing season. Extensive analysis of alkaline phosphatase activity has not been conducted in Boomer Lake. On three sampling dates in 1982 and 1984, alkaline phosphatase activity ranged between 0.04 and 0.4 $nmol \cdot L^{-1} \cdot min^{-1}$ in surface water samples, a range somewhat lower than observed in the other two lakes. In Sangre Isle Lake, an inverse linear relationship has been noted between SRP concentrations and chlorophyll-specific alkaline phosphatase activity on a seasonal basis (Francko 1983b, 1984a, 1984b).

Results

Rate of Phosphate Uptake

Phosphate was taken up by seston at similar rapid rates in each of these lakes in the summer and at similar but considerably slower rates in the autumn (Table 2). The turnover rate (*k*) and its inverse, the turnover time (*T*), also varied seasonally on each lake, being faster in the summer than in the autumn. Sangre Isle Lake showed both the greatest proportional uptake (highest *k*) and the greatest velocity of phosphate uptake at each time studied. In the autumn and in the midsummer the turnover time of available phosphate in Sangre Isle Lake was short, characteristic of P-limited communities. Phosphate turnover and uptake was slower in Sanborn Lake than in Sangre Isle Lake. Turnover of available phosphate in Boomer Lake showed the greatest seasonal variation, being similar to the other lakes in the summer but exceptionally low in the autumn.

Immediate Fate of Phosphate

Phosphate can sorb to detrital and clay surfaces or it can be assimilated by living cells. We distinguished between these possible fates by determining the particle size distribution and the bouyant density of the radiolabelled particles 24 h after adding the initial spike of [^{32}P]phosphate.

In each of the lakes, greater than 80% of the added radiolabel was retained on Millipore HA filters after several hours. Longer incubation did not increase the fraction retained, indicating that an equilibrium was established within 24 h. The size distribution of radiolabelled particles was similar in each lake (Fig. 1). Greater than 80% of the added radiolabel was associated with particles larger than 0.45 μm. At least 70% and usually greater than 90% of the radiolabelled particles were apparently smaller than 3.0 μm but greater than 0.45 μm. Only in Sanborn Lake in the summer did we observe a significantly greater retention of label on 0.2-μm filters. Only in Sangre Isle Lake in the summer was a significant portion of the label retained on 3.0-μm filters, indicating that about half of the phosphate available was probably taken up by large cyanobacterial cells which were abundant at that time. Collectively, these data support the earlier findings regarding size-selective uptake of phosphate in these lakes (Francko 1986).

Clays and silts were separated from living cells and detritus by sedimentation to isopycnic position in a non-ionic discontinuous sucrose gradient (Fig. 2). Light microscopy of the fractions removed from the centrifuge tubes showed only clay particles and a few diatom frustrules were at the bottom of the tube. Intact cells and detritus predominated at the interface between the "heavy" (1.30 $g \cdot mL^{-1}$) and the "light" (1.03 $g \cdot mL^{-1}$) sucrose solutions. Some cells either migrated very slowly through the light sucrose solution or reached an isopycnic posi-

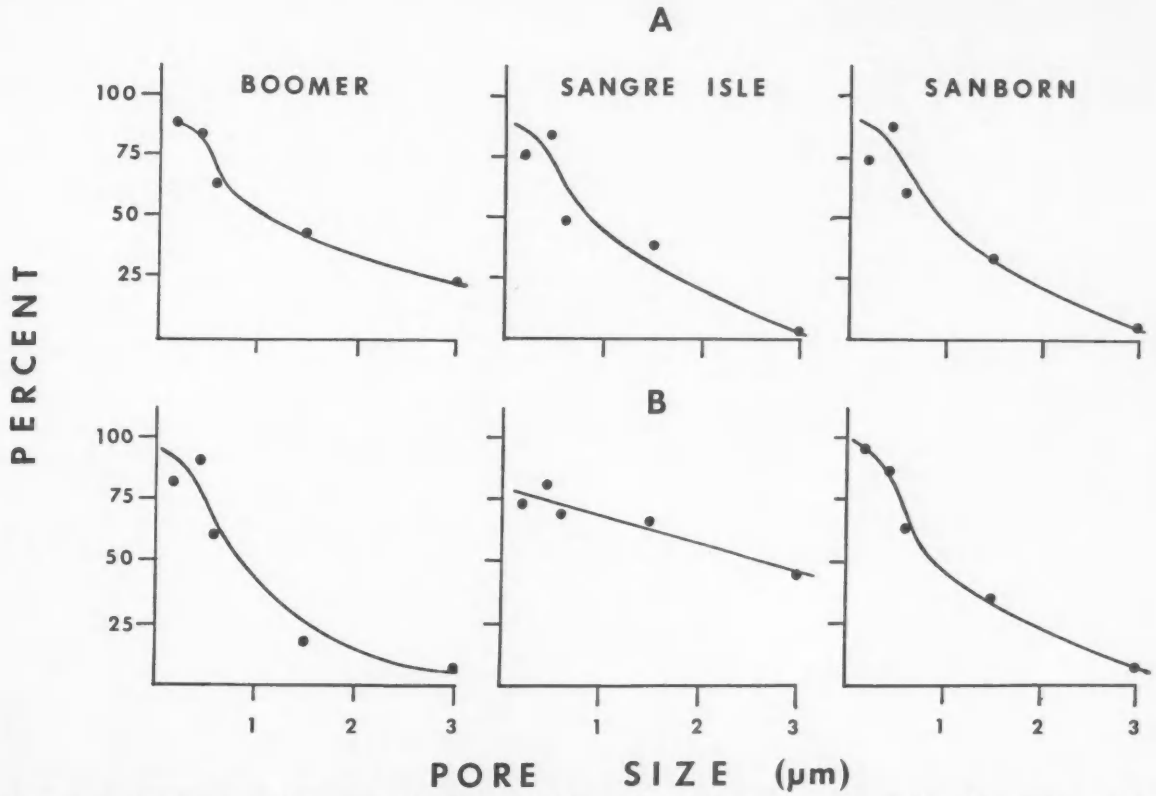

FIG. 1. Size fractionation of particles associated with [^{32}P]phosphate taken up from solution. Twenty-four hours after addition of label, particles were collected onto filters differing in pore size: 0.2, 0.45, 0.6, 1.5, and 3.0 μm. Points are means of triplicate observations. "100%" was defined as the total amount of label present in the aliquot. (A) Collection date 17 November 1983; (B) collection date 25 July 1984.

tion at a density somewhat less than 1.03 g·mL^{-1}, especially in the summer sample from Sangre Isle Lake. Pseudovacuoles in cyanobacteria present at that time may have survived centrifugal forces and caused the cells to remain bouyant (Price et al. 1978).

A greater percentage of phosphate was sorbed to high-density particles in Boomer Lake than in the other lakes (Table 2). In both Sangre Isle Lake and Sanborn Lake, at least 80% of the radiolabel taken up in 24 h was associated with particles retained at or above the density shelf. Only about 60% of the label was retained at or above the shelf in Boomer Lake.

Alkaline Phosphatase Activity

At both times that these lakes were examined, we observed the greatest amount of phosphatase activity and the greatest rate of release of phosphate from naturally occurring PME in Sangre Isle Lake, the most productive of these lakes (Table 3). Sanborn Lake samples showed less dependence on these organic P compounds, having a lower concentration of PME in the filtered water and a consistently lower phosphatase activity. Boomer Lake showed no detectable release of phosphate from PME either in the autumn or the summer.

Discussion

Contemporary views conceptualize P dynamics as a set of processes by which available P is taken up by plankton, utilized in the food chain, and eventually released either directly or by detrital mineralization, rendering unavailable forms of P once again "available." In the absence of evidence to the contrary, this P cycle is considered to be fundamentally the same for all P-limited communities (e.g. Tarapchak and Nalewajko 1986). Communities are viewed as differing quantitatively by the rates at which P moves through the various forms in this scheme, but they are not viewed as differing in the fundamental structure of the P cycle itself.

Because of the dependence on phosphate as the major if not the sole compound directly assimilated by phytoplankton and bacterioplankton, much attention has focused on characterizing the rate of phosphate uptake and its immediate fate. P-limited communities are characterized by phosphate turnover times of less than 10 min, and Rigler (1964) was among the first to report phosphate turnover times under 2 min. Lean et al. (1983), observing Lake Erie plankton communities throughout the growing season, found that P-limited communities had phosphate turnover times of 20 min or less, while P-sufficient communities had turnover times greater than 1 h. The fate of phosphate is often characterized by particle size. Many workers have noted that most of the phosphate is taken up by particles capable of passing through filters with pores 5.0 μm in diameter (e.g. Currie and Kalff 1984). Consistent with this finding, Francko (1986) reported that greater than 50% of the total sestonic ^{32}P

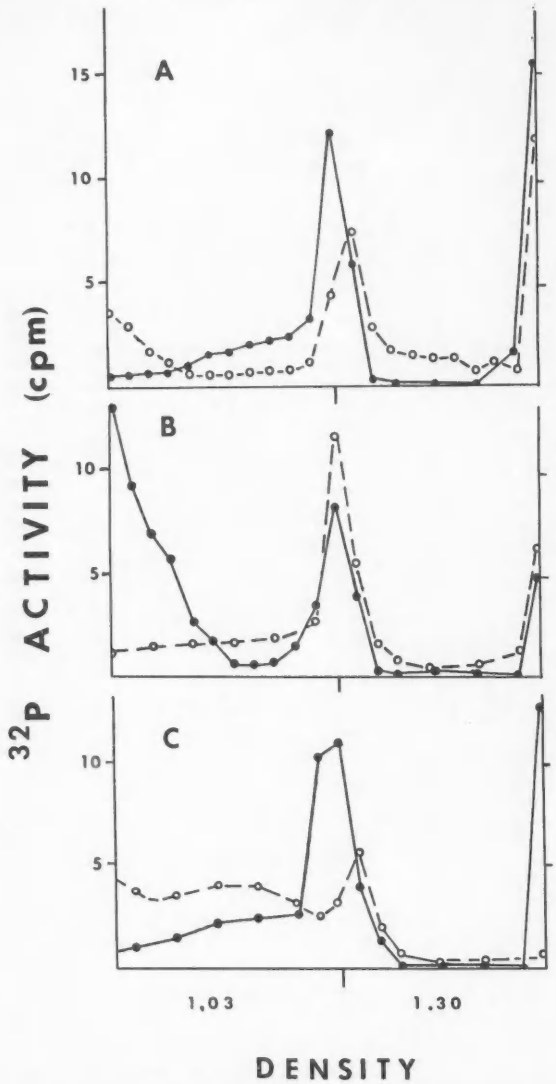

FIG. 2. Sedimentation through a discontinuous sucrose gradient (step gradient) to isopycnic position. A sucrose solution having a density of 1.03 g·mL^{-1} was layered on top of a sucrose solution having a density of 1.30 g·mL^{-1}. A sample incubated at ambient temperature for 24 h with [^{32}P]phosphate was placed at the top of the tube and the particles were sedimented to their isopycnic positions (3000 × *g*, 20 min) in a swinging bucket rotor. Collection dates: 17 November 1983 (○) and 25 July 1984 (●). (A) Boomer Lake; (B) Sangre Isle Lake; (C) Sanborn Lake.

uptake in summer waters from each of these lakes passed through 3.0-μm filters.

Phosphate uptake in a steady-state situation must be replenished concomitantly at an equal rate. The sources and processes of such a rapid replenishment of available phosphate are unknown. Because the turnover times of phosphate are generally most rapid when allochthonous inputs are small, most investigators regard internal cycling mechanisms to be most important in rapidly replenishing the available phosphate. Hutchinson (1957) considered release of phosphate from DOP compounds to be potentially the most important source of phosphate, noting that DOP is often in 10-fold excess over the available phosphate. The processes involved in recycling phosphate from DOP are not well understood. We have identified two processes by which phosphate can be released from certain DOP compounds (Francko and Heath 1979). One process is dependent on a biotic adaptation to P limitation: genetic derepression of phosphatase that in turn releases phosphate from PME. The other process involves photodependent abiotic release of phosphate from humic compounds (Francko and Heath 1983).

Elsewhere we have suggested that aquatic communities may differ fundamentally according to the processes by which P is made available to plankton from DOP (Francko and Heath 1979). Some communities may be structured to depend on biotic release of P from DOP; others that are heavily influenced by humic compounds may differ in their structure and depend on abiotic photodependent release of P from DOP compounds. Alternatively, lake communities may be arranged in a "continuum," ranging from those dominated by biotic processes at one extreme to lakes dominated by abiotic processes as the other extreme (Francko 1986). In this view, the "position" of a given lake community along the hypothetical continuum may change with time and be sensitive to certain "keystone" physical, chemical, or biological factors such as dissolved humic compounds complexed with iron (Cameron and Liss 1984).

The present study suggests that clay suspensoids may represent another important factor determining the fundamental structure of the P cycle by influencing the fate of available P. Although phosphate was rapidly taken up by seston of similar size in each of these lakes, its immediate fate in Boomer Lake differed from that in the other lakes. Much of the phosphate that could have gone to biota was apparently sorbed to clay suspensoids, instead. Phytoplanktonic productivity in P-limited lake communities impacted heavily by siltation may become dependent on sorption–desorption equilibria and be readily influenced by factors that affect those equilibria and the kinetics of release of phosphate from clay surfaces.

The mechanisms of phosphate adsorption and desorption from clays are not completely understood because of the complex interdependence of the many reactions involved and the different behaviors of various mineral clays and metal oxides that may be present in aquatic systems. Clay minerals are layered structures of alternating tetrahedral and octahedral layers which may permit varying degrees of penetration of water-soluble ions depending on whether the clay can expand in water (e.g. montmorillonites) or not (e.g. illites) (Stumm and Morgan 1981). Bar-Yosef and Kafkafi (1978) observed that both the adsorption and desorption of phosphate on kaolinite suspensions showed biphasic kinetics: a rapid first-order and a slow first-order reaction. Similar biphasic kinetics of phosphate uptake and desorption from goethite (a-FeOOH) were observed by Madrid and Posner (1979), and may be influenced by the aggregation of goethite colloids resulting in phosphate burial and slow phosphate release kinetics (Anderson et al. 1985).

The adsorption of phosphate can be viewed as the formation of a surface-limited solid solution where the effective surface is influenced by the concentration of phosphate (Miller et al. 1986). Temperature, pH, and ionic strength of the water affect the equilibria and the kinetics of these reactions (Stumm and Morgan 1981). Complicating the situation further are the effects of competitive reactions with other ions dissolved in the water or in the solid phase (Roy et al. 1986). Also, organic materials

TABLE 3. Rate of phosphate release from phosphomonoesters.

Date/lake	Phosphomonoester concentration ($\mu g \cdot L^{-1}$)	Phosphomonoester concentration ($nmol \cdot L^{-1}$)	Phosphatase activity ($nmol \cdot L^{-1} \cdot min^{-1}$)	Velocity of phosphate release ($nmol \cdot L^{-1} \cdot min^{-1}$)
17 November 1983				
Boomer	0.8	25.8	Undetectable	—
Sangre Is.	6.2	200.0	2.25	0.270
Sanborn	2.4	77.4	0.40	0.019
25 July 1984				
Boomer	Undetectable	—	1.30	—
Sangre Is.	5.7	183.9	20.20	2.229
Sanborn	3.8	122.6	1.40	0.103

may coat the clays and greatly influence both the extent and rate of phosphate adsorption (Stumm and Morgan 1981).

Besides the influence that heavy siltation may have on our lake communities, this study also suggests that the relative importance of phosphatase as a phosphate-replenishing mechanism may have differed among these lakes. The relative importance of phosphate regeneration from PME can be determined by comparing the potential rate of phosphate release from PME with the rate of phosphate uptake by seston. We observed that phosphatase had the greatest apparent importance in the eutrophic lake (Sangre Isle Lake) but much less apparent importance in oligotrophic Sanborn Lake. Phosphatase-mediated regeneration of phosphate exhibited no apparent importance in Boomer Lake at the times that we observed it, although small amounts of phosphatase activity have been seen in this lake in earlier studies. This finding suggests that the P cycle in Boomer may have a different structure than that generally envisioned in lakes not heavily impacted by siltation. Whether this apparent unimportance of DOP in the P dynamics of Boomer Lake is a direct effect of siltation (e.g. by sorption of DOP onto clay surfaces) is unknown.

We suggest that the presence of clay suspensoids in lakes and artificial impoundments may have a profound effect on the community, not only by light attenuation but also by restructuring the P dynamics. The planktonic communities of Sangre Isle Lake and Sanborn Lake appeared to exhibit a biotically mediated P cycle in which living cells assimilate phosphate and synthesize the enzymes necessary to maintain the availability of P. The community of Boomer Lake on the other hand appeared to exhibit a P cascade instead of a cycle, a situation in which plankton competed with clays for available phosphate and where DOP appeared to play little role, at least at the times examined. Whether these differences are due solely to the presence of clay suspensoids is uncertain. The potential significance for rendering P availability sensitive to those factors that influence the extent and rate of phosphate sorption processes on clay minerals and metal oxide suspensions deserves further attention.

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NOTES

Osmoregulatory Ability of Wild Coho Salmon (*Oncorhynchus kisutch*) and Dolly Varden Char (*Salvelinus malma*) Smolts

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Osmoregulatory ability of wild coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvelinus malma*) smolts migrating from a small stream in southeastern Alaska was assessed by plasma Na^+ levels after a 24-h seawater challenge. Osmoregulatory ability of coho salmon was unaffected by time of out-migration, water temperature, and fish size. Osmoregulatory ability of Dolly Varden char was apparently affected by time of out-migration or water temperature but not by fish size. Char migrating in the first half of the migration period, when water temperature was usually <8.0°C, had lower plasma Na^+ levels than did char migrating in the second half when temperatures were ≥8.0°C. A plasma Na^+ threshold of 170 mmol·L^{-1}, used by others to separate smolts from silvery parr, indicated that 70% of the coho salmon and 80% of the Dolly Varden char we sampled were physiologically prepared to enter seawater. The remaining fish may have suffered some level of osmoregulatory stress.

Nous avons mesuré la capacité d'osmorégulation chez des smolts sauvages de saumon coho (*Oncorhynchus kisutch*) et de Dolly Varden (*Salvelinus malma*) provenant d'un petit cours d'eau du sud-est de l'Alaska en mesurant les niveaux de Na^+ dans le plasma après une exposition de 24 h à l'eau de mer. La capacité d'osmorégulation du coho n'était pas touchée par le moment de la migration, par la température de l'eau ou par la taille des poissons. Dans le cas de la Dolly Varden, cette capacité semblait modifiée par le moment de la migration ou par la température de l'eau mais pas par la taille des poissons. Les Dolly Varden qui migraient dans la première moitié de la période de migration, alors que la température de l'eau était généralement de <8,0°C, présentaient des concentrations de Na^+ dans le plasma inférieures à celles des poissons qui migraient dans la deuxième moitié, alors que les températures étaient de ≥8,0°C. En fonction d'un seuil de 170 mmol·L^{-1} de Na^+ dans le plasma, utilisé par d'autres auteurs pour distinguer les smolts des tacons, 70 % des cohos et 80 % des Dolly Varden échantillonnés étaient physiologiquement préparés à entrer dans l'eau de mer. Les autres poissons souffraient peut-être d'un certain stress au niveau de l'osmorégulation.

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Visual identification of functional smolts is difficult in wild populations of anadromous salmonids because some parr have morphological characteristics similar to smolts (body silvering, fin darkening). One way to identify smolts is by the increase in osmoregulatory ability (i.e. the ability to excrete salts and thereby maintain the plasma at one-third the osmotic concentration of seawater) which preadapts fish to life in seawater (Wedemeyer et al. 1980). During the critical period when they first enter seawater, fish with good osmoregulatory ability are more likely to survive (Clarke and Blackburn 1977), have better growth and swimming ability (Houston 1959; Clarke and Blackburn 1978; Flagg et al. 1983), and may be less susceptible to predation.

Osmoregulatory ability has been studied in juvenile salmonids during parr–smolt transformation to determine the optimum release time for hatchery-reared smolts (Clarke and Blackburn 1977, 1978). Few studies, however, have been done on the physiological ability of wild fish to adapt to seawater. The objectives of our study were to (1) determine the timing and magnitude of seawater migration of wild coho salmon (*Oncorhynchus kisutch*) and Dolly Varden char (*Salvelinus malma*) smolts from a small stream in southeastern Alaska, (2) determine the osmoregulatory ability of smolts by measuring plasma Na^+ after a 24-h seawater challenge, and (3) relate changes in plasma Na^+ to time of migration.

Study Site

The study site is a third-order stream, informally called Deer Track Creek (56°N, 133°W), on the northwestern part of Prince of Wales Island in southeastern Alaska. The watershed area is 1.5 km^2, mean channel width is 3.5 m, and summer discharge is 0.05 $m^3 \cdot s^{-1}$. The intertidal area at the stream mouth is short (20 m) and steep.

Methods

Seaward migrants were collected from 8 April to 9 June 1984 at a fence trap (Argue et al. 1979) approximately 170 m upstream from seawater. We measured fork length (FL) of all fish captured and weighed a subsample of the fish throughout the migration period. Periodically, a maximum of six coho salmon per day (selected to cover the range of sizes in the daily catch) were tested for osmoregulatory ability from 3 to 24 May. One to four Dolly Varden char also were tested periodically from 23 April to 26 May, primarily during the early and late parts of the migration period. To reduce stress, fish tested for osmoregulatory ability were removed from the trap promptly each day. Stream temperature and discharge were measured daily throughout the migration period.

Osmoregulatory ability of fish was assessed by measurement of plasma Na^+ after a 24-h seawater challenge test (Clarke and Blackburn 1977). No more than six fish were placed in each 132-L plastic container filled with synthetic seawater (28–32‰). The container was placed in the stream to maintain ambient temperature and was aerated with a battery-powered aerator. Fish were killed after 24 h, their tails cut off, and blood was collected into a microhematocrit tube treated with ammonium-heparin. Blood was transferred to a 1.5-mL plastic tube and centrifuged for 3–4 min. Plasma was then removed and stored frozen at 0°C for up to 3 mo before analysis. No fish died or appeared moribund after seawater challenge.

Plasma Na^+ levels were determined for 51 coho salmon and 31 Dolly Varden char by an Orion 1020 Na^+–K^+ analyzer on plasma samples diluted with deionized water to 33%. Plasma from eight coho salmon and eight char was insufficient for individual analysis and samples were pooled according to fish similar in size and date of capture, resulting in 47 coho salmon and 27 char for analysis. Plasma Na^+ values from the Orion analyzer were converted to equivalent values on a flame spectrophotometer with a conversion coefficient of 0.941 (conversion chart, Orion Research Inc., 840 Memorial Dr., Cambridge, MA 02139). To use this conversion coefficient, plasma protein and lipid concentrations were assumed to be similar for both species. This conversion coefficient was based on previously reported plasma protein and lipid values determined for coho salmon by Wedemeyer and Chatterton (1971) and Woo et al. (1978).

To test whether osmoregulatory ability of coho salmon differed by time of migration, we compared mean plasma Na^+ values during four equal time intervals in May. We also tested for improved osmoregulatory ability over time, via a test for trend by polynomial contrasts (Winer 1962). Dolly Varden char were not collected by equal time intervals throughout the migration period; therefore, to test whether their osmoregulatory ability differed by time of migration, mean plasma Na^+ levels were compared in fish sampled in the first and second half of the migration period. Product-moment correlation coefficients (Zar 1974) were used to determine whether osmoregulatory ability was correlated with fish size or water temperature.

Results

The seaward migration of juvenile coho salmon began in late April, peaked on 18 May, and ended by early June (Fig. 1). A total of 657 coho salmon were captured at the trap; all fish had the morphological appearance of smolts. The migration peak was coincident with rising streamflow during the first major freshet of spring (Fig. 1). Most (81%) fish migrated when water temperature was ≥9.0°C.

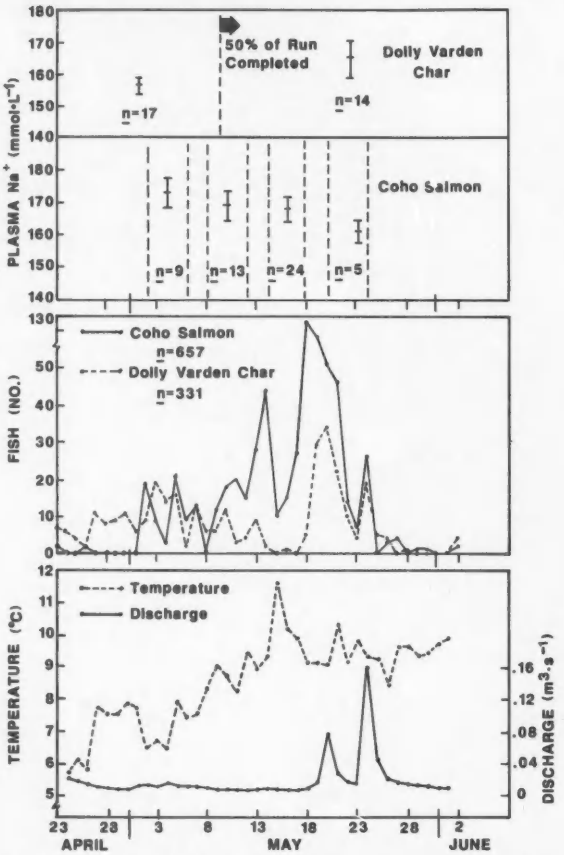

FIG. 1. Plasma Na^+ level (mean ± SE) and numbers of coho salmon and Dolly Varden char out-migrating in 1984 from Deer Track Creek on Prince of Wales Island in southeastern Alaska. Plasma Na^+ concentration within intervals represents mean date of sampling. Water temperature and discharge data were recorded during the smolt migration period.

Sizes of coho salmon used in the seawater challenge were as follows: FL ranged from 86 to 133 mm ($\bar{x}$ = 104 mm, SE = 1.4) and weight ranged from 6.4 to 18.8 g ($\bar{x}$ = 10.5 g, SE = 0.4). Plasma Na^+ levels ranged from 144.5 to 206.4 $mmol \cdot L^{-1}$ ($\bar{x}$ = 168.0 $mmol \cdot L^{-1}$, SE = 2.4); levels were ≥200 $mmol \cdot L^{-1}$ for 10% of the fish sampled. Coho salmon captured and not challenged in seawater were similar in size ($\bar{x}$ FL = 102 mm).

Plasma Na^+ levels of coho salmon were not significantly (P = 0.63, F-test) different between time intervals (Fig. 1). An improved trend in osmoregulatory ability with time was observed but was not statistically significant (P = 0.22). Small sample size in the first, and especially the last, sampling interval may be responsible for this result. Furthermore, plasma Na^+ of coho salmon was not significantly ($P > 0.05$) correlated with fish length ($r = -0.11$), weight ($r = -0.16$), or water temperature ($r = -0.10$).

The seaward migration of Dolly Varden char began in late April, peaked on 20 May, and ended by early June (Fig. 1). A

total of 331 char were captured at the trap; 95% had the morphological appearance of smolts. About half of the char migrated prior to 10 May at water temperatures usually <8.0°C.

Sizes of Dolly Varden char used in the seawater challenge were as follows: FL ranged from 107 to 148 mm ($\bar{x}$ = 121 mm, SE = 1.9) and weight ranged from 9.0 to 23.5 g ($\bar{x}$ = 13.3 g, SE = 0.7). Plasma Na^+ levels ranged from 128.7 to 203.5 $mmol \cdot L^{-1}$ ($\bar{x}$ = 161.0 $mmol \cdot L^{-1}$, SE = 2.9); levels were ≥200 $mmol \cdot L^{-1}$ for 6% of the char sampled. Char captured and not challenged in seawater were similar in size ($\bar{x}$ FL = 123 mm). All char used in the seawater challenge test had the morphological appearance of smolts.

Char migrating in the first half of the migration period had significantly ($P \leq 0.05$, *t*-test) lower plasma Na^+ than those in the second half (Fig. 1). Char had significantly ($P < 0.05$, *t*-test) lower plasma Na^+ ($\bar{x}$ = 154.3 $mmol \cdot L^{-1}$, SE = 3.0, N = 15) at water temperatures <8.0°C than at ≥8.0°C ($\bar{x}$ = 167.2 $mmol \cdot L^{-1}$, SE = 4.5, N = 16). Osmoregulatory ability of char was not significantly correlated with fish length (r = 0.15) or weight (r = −0.01) but was significantly ($P \leq 0.05$) correlated with water temperature (r = 0.36). As water temperature rose continually from 6.5°C on 4 May to 11.6°C on 15 May, a steady decline was observed in total number of char captured. The peak water temperature of 11.6°C coincided with an almost complete halt in Dolly Varden char migration for 3 d (Fig. 1).

Discussion

After seawater challenge, plasma Na^+ levels of wild coho salmon in our study and in hatchery-reared coho salmon in British Columbia (Clarke 1982) were similar. Clarke and Blackburn (1977) demonstrated that a plasma Na^+ level of 170 $mmol \cdot L^{-1}$ after seawater challenge is the threshold that separates functional hatchery smolts from silvery parr. Laboratory tests (Clarke and Blackburn 1977) have shown that after transfer to seawater, fish with plasma Na^+ below 170 $mmol \cdot L^{-1}$ grow rapidly whereas other studies (Houston 1959; Clarke and Blackburn 1978) have shown that those fish with elevated plasma Na^+ grow slowly and have impaired locomotion. Of the coho salmon we sampled, 70% had plasma Na^+ levels ≤170 $mmol \cdot L^{-1}$; these fish would be considered functional smolts. The remaining 30% may have suffered some level of osmoregulatory stress.

Information on osmoregulatory ability of Dolly Varden char is scarce. Our data indicate that after 24 h in seawater, char had lower plasma Na^+ than wild and hatchery coho salmon. The seawater challenge – plasma Na^+ test has been used as an indicator of smolt status for other char, brook trout (*Salvelinus fontinalis*), other Pacific salmon (*Oncorhynchus* sp.), and trout (*Salmo* sp.) (Wedemeyer et al. 1980; McCormick et al. 1985). If we assume that the 170 $mmol \cdot L^{-1}$ threshold is also applicable to Dolly Varden char, then 80% of the fish we sampled were functional smolts and 20% may have suffered some level of stress.

Ten percent of the coho salmon and 6% of the char had plasma Na^+ levels ≥200 $mmol \cdot L^{-1}$. Fish with such high plasma Na^+ suffer high mortality in seawater (Clarke and Blackburn 1977). Upon entering seawater, stressed fish may not adjust to seawater and die. Some may survive and adjust completely by selecting the lower salinities of surface waters. Gradual acclimation to seawater in an estuary has been shown to be important for the eventual seaward migration and survival of brook trout (McCormick et al. 1985).

Size, time of migration, and water temperature did not affect osmoregulatory ability of coho salmon. Clarke (1982) also found that size did not affect performance in seawater. Water temperature (5–12°C) in our study was within the range described by Zaugg and McLain (1976) for sustained development of coho salmon smolts in laboratory tanks. Most coho salmon in our study migrated at water temperatures of 9–12°C, similar to temperatures at time of migration of coho salmon smolts in an Oregon stream (Rodgers et al. 1987).

Time of migration or water temperature may have affected the osmoregulatory ability of char. Plasma Na^+ levels of char were lower in the first half of the migration period when water temperatures were usually <8.0°C; therefore, char migrating at these temperatures probably were more physiologically prepared to enter seawater than those char migrating in the second half of the migration period when temperatures were ≥8.0°C. The roles of temperature and time are confounded in the apparent decreased osmoregulatory ability of char in the second half of the migration period, although evidence may favor a temperature effect. Elevated water temperatures are known to inhibit smolt development (Wedemeyer et al. 1980), especially in steelhead trout (*Salmo gairdneri*) (Zaugg and Wagner 1973). Armstrong (1970) reported that the downstream migration of Dolly Varden char in an Alaskan stream was mostly completed when water temperature warmed to 6°C. Whether or not char migrating in the first or second half of the migration period would ultimately show differences in survival to adulthood is unclear at this time; plasma Na^+ levels of fish migrating in the second half were still below the 170 $mmol \cdot L^{-1}$ threshold. In addition, differences in plasma Na^+ of early and late migrants may have been affected by the length of the seawater challenge. Blackburn and Clarke (1987) suggested that a different test period may apply for cold versus warm water.

Low streamflow, especially in the small tributaries and headwaters of the watershed where Dolly Varden char were common, may have inhibited some char from migrating earlier in spring. Rainfall in April and May was approximately 14 cm, 25% lower than the 30-yr mean, with most of the rain falling during the second half of May (NOAA 1984a, 1984b). Evidence that migration of some fish may have been delayed occurred 15–17 May (Fig. 1) when few fish, if any, passed through the weir. Fish remaining in the stream during that time were exposed to water temperatures as high as 11.6°C, which may have interfered with osmoregulatory development and may have accounted for the elevated plasma Na^+ levels of char observed during the latter part of their migration.

Because all fish (some with elevated plasma Na^+) survived after 24 h in seawater, a portion of the migrating coho salmon and Dolly Varden char may depend on a mixing zone of freshwater and seawater for acclimation. Environmental conditions, especially water temperature, may affect the osmoregulatory ability of fish. Further studies are needed to refine the association between environmental conditions, the osmoregulatory competence of seaward migrants, and subsequent marine survival rates.

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Growth and Survival of Hormone-Sterilized Coho (*Oncorhynchus kisutch*) and Kokanee Salmon (*O. nerka*) in a Lacustrine Environment

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Parkinson, E. A., and K. Tsumura. 1988. Growth and survival of hormone-sterilized coho (*Oncorhynchus kisutch*) and kokanee salmon (*O. nerka*) in a lacustrine environment. Can. J. Fish. Aquat. Sci. 45: 1490–1494.

Coho (*Oncorhynchus kisutch*) and kokanee salmon (*O. nerka*) were sterilized using 17α-methyltestosterone treatments and released into a lacustrine environment. The treatment appeared to be successful and sizes at capture were similar for treated and control fish. Return rates of hormone-treated groups of fish were much lower than those of the control groups. Differences in return rates were assumed to be due to differences in survival rates but the possibility of differential vulnerability to the capture gear could not be ruled out. The proportion of treated kokanee in the catch increased with age, indicating that treated kokanee survived beyond the normal life span of the species.

Des saumons cohos (*Oncorhynchus kisutch*) et kokanis (*O. nerka*) ont été stérilisés par des traitements à la méthyltestostérone-17α puis libérés dans un milieu lacustre. Le traitement a semblé réussir, et la taille à la capture était similaire chez les poissons traités et chez les témoins. Les taux de retour des groupes traités à l'hormone étaient nettement plus bas que ceux des groupes témoins. On pense que ces différences dans les taux de retour étaient dues à des différences dans les taux de survie, mais on ne peut éliminer la possibilité d'une vulnérabilité différente aux engins de capture. La proportion de kokanis traités dans les captures augmentait en fonction de l'âge, ce qui indique que les poissons traités survivaient au-delà de la durée normale de l'espèce.

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Sterilization of *Oncorhynchus* spp. using methyltestosterone has been suggested as a means of extending longevity and increasing the size of hatchery reared individuals captured in the fishery (Hunter et al. 1982). Captive, sterilized coho salmon (*O. kisutch*) grow and survive at near normal rates (Hunter et al. 1982) but there are little data on survival, in particular, under more natural conditions.

Survival, relative to a control group, is difficult to estimate in the ocean. The most practical means of recovering fish is through the commercial and sports fisheries as part of a larger coded wire tag recovery program. Spatial segregation of the two groups, as well as other behavioral differences, may make one group more vulnerable to fishing gear. This is especially true in the case of sterilized coho which are not expected to make a spawning migration and are therefore not expected to appear in the terminal fishery or at the hatchery. To avoid these difficulties, we compared the growth and survival of hormone-treated and normal coho in a land-locked lake instead of their normal marine environment. In addition, we also compared growth and survival of treated and untreated kokanee, the non-anadromous form of *O. nerka*, in their normal lacustrine environment.

Methods

Eggs were obtained from the Capilano Hatchery near Vancouver, B.C. (coho), and from Mission Creek on Okanagan Lake, B.C. (kokanee). Fry were sterilized using methods similar to those of Hunter et al. (1982). At 100% hatch, fry were dipped in a 200 μg/L solution of 17α-methyltestosterone (MT) for 2 h at 9.5°C. This treatment was repeated 7 and 14 d after hatching. Fry were fed every 30 min with a commercially prepared diet (BioDiet, Bio Products, Warrenton, OR). Treated groups were fed a treated diet for their first 90 d of feeding. The treated diet was prepared by spraying MT dissolved in ethanol over the food at low (3 mg MT/kg food), medium (5 mg/kg), and high (9-10 mg/kg) dosages. Treatments, size, dates, and locations of release are given in Table 1. The water temperature during both incubation and rearing was 9.5°C.

Different release groups were identified with distinctive fin clips except for the 1984 control group in Salsbury Lake (Table 1). Since there were no kokanee in this lake prior to the 1983 introduction, unclipped fish of the appropriate age were assumed to be from this group. Less than 5% of the Blue Lake, Till Lake,and 1982 brood Salsbury Lake recoveries consisted of unclipped fish. Ages were determined from scales using the method of Tesch (1968).

All of the study lakes contained introduced rainbow trout (*Salmo gairdneri*) and none contained either kokanee or coho. Other characteristics are given in Table 2.

Fish were captured in gangs of monofilament gillnets made up of 25-, 37-, 51-, 64-, and 76-mm stretched mesh.

Results

Histological examination of the 1982 brood of kokanee at the time of release indicated that the hormone treatment was effective in inducing sterilization. Gonads from the high-treatment group were subnormal in size and appeared vacuolated or cystic in most cases. In the low-treatment group, gonads were either atrophied or appeared to be in the process of resorption. Almost all of these appeared to be male in a very advanced state of development towards mature spermatozoa on a cellular level, but the testes were of subnormal size for fish of this size. On the basis of these results the 1983 brood of kokanee was given an intermediate level of hormone treatment.

Noticeable effects on external features were present in the treated groups of kokanee. In the low-treatment group, many fish were darkened and a few expressed abnormal jaw development. In the high-treatment group, many fish had undershot, overshot, or twisted lower jaws and a few exhibited spawning coloration of varying intensity. External features of coho were not systematically examined.

Size at capture — Treated fish tended to be smaller at capture than the corresponding control group, but all but one of these differences are nonsignificant (t-test, $p > 0.05$; Table 3). Within a group, mature fish were always slightly larger than immature fish.

Relative survival — Interpretation of the relative survival data is complicated by the maturation process in the control fish. Both species mature and spawn in September or October and die following spawning. At this time, mature fish may be more vulnerable to capture by gill nets because of behavioral differences such as an active search for spawning habitat. Mission Creek kokanee normally mature at age 3+ but many matured at age 2+ in Salsbury Lake and age 1+ in Blue Lake, and consequently, no prematuration sample is available for the 1983 brood in Blue Lake.

The 1984 brood of coho in Till lake was sampled at age 1+ in September of 1986 and at age 2+ in August 1987. Although

TABLE 1. Treatments, location, date, size, fin clips, and numbers released for each release group. Clip codes are LV (left ventral), RV (right ventral), A (adipose), and LM (left maxillary). All fish were released as age 0+ fry.

Species	Treatment dosage (mg/kg)	Location	Release date	Size (g)	Nuamber	Clip
Coho	0	Till Lake	June 12, 1985	5.6	13 500	LV
	10			6.5	13 500	LM
Kokanee	0	Salsbury Lake	June 6, 1983	6.3	5 708	AD
	3			6.7	9 400	LV
	9			10.2	9 600	RV
	0	Salsbury Lake	May 2, 1984	5.0	4 000	No Clip
	5			6.7	4 000	ARV
	0	Blue Lake	May 24, 1984	6.5	4 000	LV
	5			8.9	4 000	ARV

TABLE 2. Characteristics of the study lakes. TDS = total dissolved solids.

Name	Lat.	Long.	Area (ha)	Mean depth (m)	TDS (mg/L)	Other species of fish	Inlets or outlets
Blue	52°20′	122°14′	32	9.8	370	Redside shiner (*Richardsonius balteatus*)	None
Salsbury	49°21′	122°16′	79	7.5	21	Cutthroat trout (*Salmo clarki*)	
Till	52°03′	122°22′	80	7.7	1150	Lake chub (*Couesius plumbeus*)	None

TABLE 3. Size at capture for the various release groups. Numbers in parentheses are standard errors. Resuits of tests for differences between control and treated groups apply to both lengths and weights (NS = not significant, $^{**}p < 0.01$).

Species	Brood year	Lake	Capture date	Treatment	*N*	Length (mm)	*N*	Weight (g)	Sign. test
Kokanee	1982	Salsbury	May 1984	Control	73	175 (0.9)	58	54.0 (0.9)	NS
				Low	9	168 (5.1)	5	48.5 (5.8)	
			Sept. 1985	Control	79	195 (1.1)	77	86.2 (1.6)	NS
				Low	2	186 (2.1)	2	64.9 (2.7)	
				High	1	185	1	74.6	
			June 1986	Control	4	210 (3.2)			NS
				Low	4	205 (2.8)			
				High	6	209 (3.6)			
			Nov. 1986	Control	3	239 (3.8)	3	155.4 (11.4)	NS
				Low	26	237 (2.5)	26	149.3 (3.9)	
				High	13	234 (3.6)	13	141.4 (5.4)	
	1983	Salsbury	Sept. 1985	Control	13	177 (1.6)	13	60.4 (1.6)	NS
				Medium	2	144 (20.5)	2	34.8 (16.7)	
			June 1986	Control	24	218 (1.9)			
			Nov. 1986	Control	54	237 (1.4)	54	151.5 (2.5)	NS
				Medium	33	234 (1.7)	33	144.9 (2.6)	
		Blue	Sept. 1985	Control	60	312 (2.8)	33	406.1 (16.5)	**
				Medium	18	286 (2.9)	14	300.0 (11.4)	
Coho	1984	Till	Sept. 1986	Control	34	244 (14.4)	34	162.1 (10.6)	NS
				High	5	242 (16.6)	5	190.5 (37.2)	
			Aug. 1987	Control	15	323 (8.3)	15	435.2 (34.9)	NS
				High	2	360 (16.5)	2	577.0 (120.0)	

sample sizes are small, control fish were recovered in higher than expected numbers relative to treated fish in both cases (Fig. 1). Some maturing fish were caught in 1986 but all of these were precocious males and the proportion of control fish is still higher than expected when these fish are removed from the sample ($p < 0.01$). The 1987 sample was taken in early August, before there were any external signs of maturation. Gonad weight at this time was <3% of body weight. Twelve of 15 control fish were females. One of the treated fish had threadlike gonads (<0.1% of body weight) and was assumed to be sterilized. The other was a maturing male with a single, abnormally shaped gonad (2.1% of body weight). The presence of maturing fish in both of the treated samples indicates that the treatment was not completely effective.

Control fish were recovered in higher than expected proportions from all three releases of kokanee (Fig. 1). In some cases (Blue Lake; Salsbury Lake, 1983 brood, September 1985), many of the control fish were mature and when mature fish are removed from the sample, sample sizes are small and the proportion of control fish is not higher than expected. Samples of age 2+ and age 3+ kokanee contain a higher proportion of treated fish than earlier samples, presumably because many of the control fish have spawned and died.

The presence of occasional mature fish in the treated groups (Fig. 1) indicates that the treatment was not completely effective. Two of the low-treatment fish captured in Salsbury Lake in November 1986 appeared to be mature males (red coloration, resorped scales, humped backs) but had the threadlike gonads characteristic of sterile fish.

Discussion

Treated groups were presumed to contain a high proportion of sterilized fish. Well-established techniques were used (Donaldson and Hunter 1982), treated fish survived beyond the normal maturation time, and histological examination at the time of release of the 1983 brood of kokanee suggested sterility.

Our results suggest that initial survival of hormone-sterilized coho and kokanee salmon, released into a natural environment, is low but that sterilized fish do survive beyond the normal spawning date and could contribute significantly to a fishery at this time. Samples taken prior to the maturation of the control groups indicated that survival of treated fish was less than 10% of control groups. Samples taken after this time consist of an increasing proportion of treated fish, presumably because many control fish have matured and died, while treated fish at this time continue to experience only the more moderate natural mortality rate.

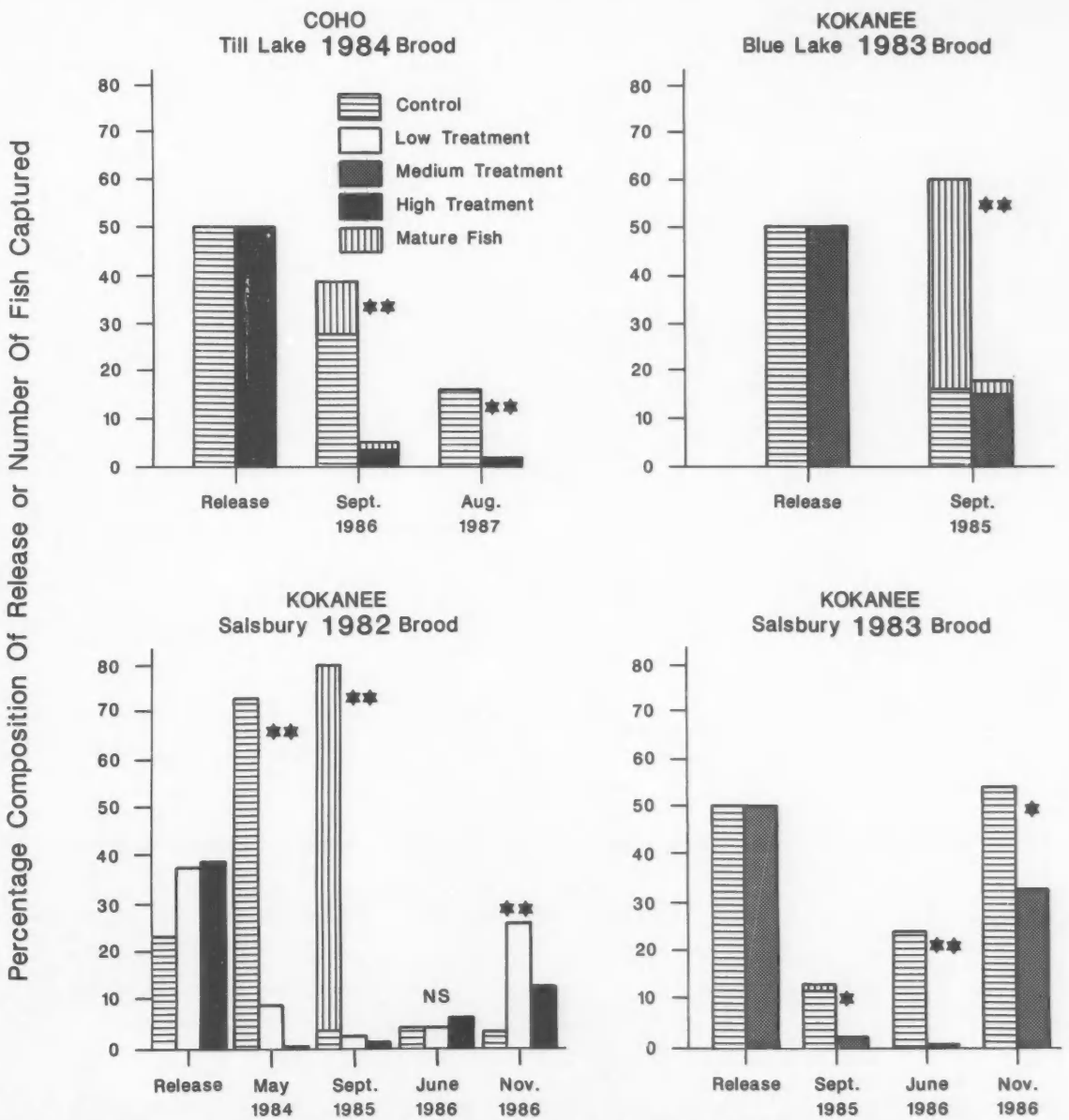

FIG. 1. Percentage composition of each release and numbers of fish captured at each date in the three study lakes. Numbers of mature fish captured are stacked on top of the appropriate group of immature fish. The significance of the difference between the proportions at the time of release and time of capture is indicated above each set of bars (chi-square test: NS = not significant, $*p < 0.05$, $**p < 0.01$).

Less extreme results were reported for treated and control coho smolts released into a marine environment (Solar et al. 1986). The return rate for a control group in the commercial and sports fisheries was 2.2 times that of the treated group. About 25% of the treated returns were age 4 and 5 fish whereas virtually all of the control returns were age 3. Treated fish that returned to the hatchery were mature, indicating that the treatment was not completely effective (I. I. Solar, 4160 Marine Drive, West Vancouver, B.C., pers. comm.). The return rate to the hatchery for the control group was 47 times that of the treated group, suggesting that a high proportion of treated fish were actually sterile (Solar et al. 1986).

MT treatment produces a variety of effects other than sterilization. Minor abnormalities in external appearance have been noted at doses less than those used in this study (Fagerlund and McBride 1977). In the amago salmon (*O. rhodurus*), MT treatment resulted in increased liver glycogen, decreased visceral lipid, and an impaired osmoregulatory ability in smolts (Miwa and Inui 1986). Smoltification was also inhibited in juvenile masu salmon (*O. masou*) treated with MT (Ikuta et al. 1985).

Despite these side effects, there is no obvious explanation for the apparent differences in survival rates between the treated and control groups. Survival of treated fish in captivity is similar to untreated fish (Hunter et al. 1982). Treated fish grow only slightly slower than controls, both in captivity (Solar et al. 1986) and in the lake environment used here, indicating that the survivors in the treated group did not have serious difficulty acquiring food in the lake environment. MT is rapidly eliminated from fish tissues (Fagerlund and Dye 1979), so survival is unlikely to be affected by high levels of MT in treated fish at the time of release. The generally larger size at release of the treated fish should have conferred a survival advantage rather than disadvantage to these groups. This difference in size at release is probably due to the action of the hormone as a growth promoter (Fagerlund et al. 1979) rather than differences in culture conditions.

It is possible that treated fish are less vulnerable to capture because of either size or behavioral differences. The small size differences between control and treated groups imply that gillnet selectivity should not be a serious problem. Behavioral avoidance is a possibility but is probably a more serious problem in the marine environment where recoveries are made in fisheries spread over hundreds of square kilometres than in the small (<100 ha) lakes used in this study.

Poor initial survival does not necessarily imply that sterilized fish are not useful in some situations. In some lakes, for example, kokanee normally mature at <200 mm and very few recruit into the sportfishery. If sterile fish survive and grow larger than this size, a high initial mortality could be tolerated if surplus eggs are available from another stock.

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Error in Estimation of Feeding Rates from Changes in Mean Body Mass

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Because of nonlinear relationships between body mass and many parameters in energetics models, rates of food intake calculated from change in mean body mass (a typical application of energetics models) do not necessarily equal true mean rates of food intake calculated from individual changes in body mass. Using both hypothetical data and actual field data for largemouth bass (*Micropterus salmoides*) marked with individually numbered tags, we show that discrepancies increase with variability in body mass but are negligible (<3.5%) in all cases examined. Biased estimates of change in mean body mass, such as might result from size-selective mortality or sampling gear, can lead to substantial errors in energetics model estimates of mean food consumption rates. Use of growth data for individually marked fish in conjunction with an energetics model permits calculation of confidence intervals, statistical comparison of food consumption rates, and examination of relationships between foraging success and individual body mass.

À cause des rapports non-linéaires entre la masse du corps et beaucoup de paramètres dans les modèles d'énergie, les taux de l'adduction de la nourriture calculés du changement dans la masse moyenne du corps (une application

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typique de modèles d'énergie) ne sont pas nettement pareils aux taux moyens véritables de l'adduction de la nourriture calculés du changement individuel dans la masse du corps. En employant les deux données hypothétiques et les données véritables pour des achigans à grande bouche (*Micropterus salmoides*) marqués avec des étiquettes numérotées individuelles, nous montrons que les différences augmentent avec la variabilité dans la masse du corps mais elles sont négligeables (<3,5%) dans tous les cas examinés. Les évaluations préjugées du changement dans la masse moyenne du corps, comme peut sorter de la mortalité sélective à cause de la taille ou de l'appareil choisi, peuvent causer des défauts substantiels dans les évaluations des modèles d'énergie des taux moyens de l'adduction de la nourriture. L'emploi des données de croissance pour les poissons marqué individuel en conjonction avec un modèle d'énergie permet le calcul des intervalles de confiance, la comparaison statistique des taux de l'adduction de la nourriture, et l'examen des rapports entre le succès en fourragant et la masse du corps individuel.

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Use of fish energetics models has burgeoned in recent years with the proliferation of individual species models (e.g. Kitchell et al. 1977; Kitchell and Breck 1980; Rice et al. 1983), the favorable comparison of energetics model predictions with independently derived field estimates (Rice and Cochran 1984), and, finally, the bona fide use of energetics models to answer ecological questions (Stewart et al. 1981; Lyons and Magnuson 1987). Because energetics modeling only recently has been applied to field situations, there is a need to define appropriate guidelines for its use. Bartell et al. (1986), for example, examined the importance to model predictions of variation in internal model parameters and in external variables, and they argued that sensitivity analyses should be regarded as necessary to the development and application of energetics models to ecology.

Perhaps the most useful and appropriate application of an energetics model is to estimate food consumption, which is difficult to measure directly, from change in body mass, which is relatively easy to measure. Rice and Cochran (1984) showed that such estimates may be inaccurate if the interval between samples is too great, especially when food resources are temporally dynamic. Other concerns, which we address here, involve the use of mean body mass.

First, change in body mass typically is estimated from the difference in mean values at the beginning and end of an interval; variability about the mean values typically is ignored. However, because of nonlinear relationships between body mass and many parameters in energetics models, rate of food intake calculated from change in mean body mass will not necessarily equal a mean rate of food intake calculated from individual changes in body mass. Any discrepancy is likely to increase with increasing variability about mean body mass. In an analogous case, Nielsen and Schoch (1980) found that estimation of mean weight from mean length resulted in errors of up to nearly 90% of actual mean weight, with error increasing as a function of the exponent of the length–weight relationship and the coefficient of variation in length.

Any error in estimates of mean body mass will also contribute to error in resulting estimates of food consumption. In particular, size-selective mortality during an interval of interest, or use of size-selective sampling gear, may result in biased estimates of change in mean body mass (Ricker 1975). For example, if small fish experience relatively greater mortality during an interval, then use of change in mean body mass of the survivors should lead to overestimation of mean individual feeding rates.

Here, we assess the error inherent in using an energetics model to estimate food consumption rates from changes in mean body weight. We also suggest the utility of using data for individually tagged fish in conjunction with energetics modeling.

Methods

We used Hewett and Johnson's (1987) version of the energetics model for largemouth bass (*Micropterus salmoides*) first described by Rice et al. (1983) and further analyzed by Cochran and Rice (1982), Rice and Cochran (1984), and Bartell et al. (1986). Three sets of modeling exercises were conducted. In all cases, an important model parameter is P, the proportion of the maximum possible daily food consumption that is actually eaten, where the maximum possible consumption depends on body mass and temperature (Kitchell et al. 1977).

The first set of model simulations was purely hypothetical. Three symmetric, approximately normal frequency distributions of initial body mass of 500 bass were constructed using weight intervals of 5 g. The means of the three distributions were identical (200 g), but the standard deviations varied (5.3, 10.4, 17.8 g). Three similar distributions were centered at a mean initial body mass of 50 g. The energetics model was used to simulate growth by all individuals for 30 d with P set at 0.5. Water temperature was held constant at 27.5°C, the optimum for consumption and growth (Rice et al. 1983), and the caloric densities of both bass and their prey were assumed to equal 1200 cal·g^{-1}. The distribution of final body mass corresponding to each initial distribution was used to calculate a mean final body mass. Finally, the energetics model was used to calculate the P value that would result in the observed change from initial to final mean body mass (this approach is analogous to a typical application of the model to field data), and that P value was compared with the actual P value of 0.5. Corresponding measures of cumulative food consumption over the 30 d were also compared.

The second analysis involved actual field data for largemouth bass in Tuesday Lake, Gogebic County, Michigan (Carpenter et al. 1987). Prior to stocking of bass in May 1985, Tuesday Lake contained large populations of northern redbelly dace (*Phoxinus eos*), finescale dace (*P. neogaeus*), and central mudminnows (*Umbra limi*), but no piscivores. Initial and final body mass were obtained for bass marked with individually numbered tags on 20–23 May 1985 and recaptured 28–29 June (16 individuals) and 14 September (9 individuals) of the same year. The energetics model was used to calculate a P value for each individual and to estimate its total food consumption. For each

TABLE 1. Initial and final body mass, P values, and total food consumption for (a) hypothetical simulations with P set at 0.5 and (b) actual field data for individually tagged bass from Tuesday Lake. For P values and food consumption, true means of values for individual fish are compared with energetics model estimates derived from change in mean body mass. In some cases, numbers were not rounded off so that the insignificance of differences among values could be appreciated.

	Initial body mass (g)		Final body mass (g)		P		Food consumed (g)	
N	Mean (SD)	Range	Mean (SD)	Range	True mean (SD)	From change in mean mass	True mean (SD)	From change in mean mass
				a. Hypothetical data				
500	200 (5.33)	185–215	282.58 (6.79)	263–302	0.5 (0)	0.49998	198.892 (3.396)	198.898
500	200 (10.42)	170–230	282.54 (13.22)	244–321	0.5 (0)	0.49985	198.848 (6.635)	198.836
500	200 (17.77)	160–240	282.52 (22.61)	231–333	0.5 (0)	0.49979	198.734 (11.330)	198.808
500	50 (5.33)	35–65	83.44 (7.64)	62–105	0.5 (0)	0.49975	82.716 (5.523)	82.768
500	50 (10.42)	20–80	83.32 (14.95)	39–125	0.5 (0)	0.49900	82.409 (10.890)	82.607
500	50 (17.77)	10–90	82.97 (25.65)	22–139	0.5 (0)	0.49619	81.538 (19.007)	82.007
				b. Tuesday Lake data				
May–June 16	182.5 (81.6)	87–300	216.9 (86.2)	116–335	0.61 (0.11)	0.59	57.33 (16.05)	57.95
May–September 9	208.3 (87.0)	100–330	290.3 (95.4)	153–392	0.43 (0.08)	0.42	117.20 (31.81)	118.94

of the two mark–recapture intervals, the means of these values were compared with the P value and cumulative consumption calculated from change in mean body mass of the tagged fish. The thermal regime and diet composition used as model input were derived from 1985 field data for Tuesday Lake.

To simulate the effect of size-selective mortality, we modified the distributions of final body mass resulting from the hypothetical simulations described above. Mean final body mass was recalculated after exclusion of the lower 5, 10, 25, and 50% of the distributions. These "biased" means were used along with the initial mean mass of 200 g to calculate corresponding P values and estimates of cumulative food consumption.

Results

As expected, means of P values and cumulative food consumption for individual fish were not identical to those derived from changes in mean body mass (Table 1). However, discrepancies in all cases were of such small magnitude (<1% for the hypothetical simulations and <3.5% for the Tuesday Lake data) that they would be overshadowed by any errors due to errors in estimates of mean body mass.

Several aspects of model performance were revealed by the simulations of hypothetical populations (Table 1a). Under the conditions of the simulation (e.g. $P = 0.5$), a 200-g bass is predicted to attain a final mass of 282.59 g. This is not identical to the final means attained by populations with distributions of initial body mass symmetrical about a mean of 200 g (Table 1a) because the distributions of final body mass are slightly asymmetrical about the modal weight achieved by 200-g fish. As expected, the resulting discrepancies in P values, although small, increase with the standard deviations of initial body mass. Moreover, distributions of final body mass are characterized by increased variance with respect to corresponding distributions of initial body mass. This is purely an effect of the mechanics of the model but mimics what is observed in actual populations (e.g. Keast and Eadie 1985).

When energetic processes are expressed as functions of body mass, nonlinearity is most evident at low body mass (Rice et al. 1983). We expected, therefore, that discrepancies between true means and model estimates of P values and food consumption would be greater for bass starting at 50 g relative to those starting at 200 g. Indeed this was the case (Table 1a), but the discrepancies were not large. Other trends, as discussed above, were similar for both size classes.

Further insights into model performance resulted from the use of field data for individually tagged bass from Tuesday Lake. P values calculated from the change in mean mass of tagged fish were not identical to the means of P values calculated for individual fish but fell well within confidence intervals (Table 1b). Within sampling periods, P values for individual fish were negatively correlated with initial body mass (for 22 May – 28 June, $r = 0.530$, $P < 0.05$; for 22 May – 14 September, $r = 0.514$, $P < 0.20$). P values for the period 22 May – 28 June were greater than those for the period 22 May – 14 September (ANCOVA, $F_{1,22} = 7.535$, $P < 0.025$). This apparently reflects the depletion of forage fishes through predation by bass and a habitat shift by remaining minnows into inaccessible peripheral habitats (Carpenter et al. 1987), and it under-

scores the observation by Rice and Cochran (1984) that frequent intraseasonal samples may be necessary to adequately describe predator–prey dynamics with energetics models.

Simulated biases in mean final body mass resulted in errors in estimates of *P* values and total food consumption more substantial than those due solely to the use of change in mean body mass. Starting with the most narrow distribution of initial body mass in Table 1a (mean = 200 g, SD = 5.33 g) and eliminating the lower 5, 10, 25, and 50% of the final distribution resulted in overestimates in *P* values of 0.48, 0.81, 1.80, and 3.28%, respectively, and overestimates in cumulative food consumption of 0.57, 0.97, 2.15, and 3.92%. Errors were greater in conjunction with the widest distribution of initial body mass in Table 1a (mean = 200 g, SD = 17.77 g). Eliminating the lower 5, 10, 25, and 50% of the final distribution resulted in overestimates in *P* values of 1.53, 2.84, 6.29, and 11.75%, respectively, and overestimates in cumulative food consumption of 1.83, 3.39, 7.57, and 14.27%.

Discussion

Using change in mean body mass to estimate food consumption rates with energetics models seems justified in light of the small error that is entailed. This conclusion is bolstered by our two analyses, which involved slightly different approaches. In the first analysis, all individual fish consumed food at a constant proportion of the maximum possible rate. In the second analysis, this proportion varied among individual fish. Error of the magnitude revealed by these analyses is likely to be overshadowed by sampling errors and/or biases in collecting and measuring fish.

Biases in estimates of change in mean body mass may result from size-selective mortality during the interval of interest or from use of size-selective sampling gear (Ricker 1975). Our results indicate that such biases can lead to substantial errors in energetics model estimates of food consumption rates, but in our hypothetical examples, substantial errors were observed only when size-selective effects were intense. Furthermore, in our hypothetical examples, individual fish of all sizes were assigned identical *P* values. In real situations, small fish typically grow faster than large fish. If individual *P* values were negatively correlated with body mass, as in Tuesday Lake, then the effect of size-selective mortality of smaller fish would be (1) a positive shift in final mean body mass and (2) removal of individuals with above average *P* values. Effects (1) and (2) would tend to counteract.

How do our simulated populations compare with real populations? Standard deviations of initial body mass (Table 1a) were comparable with those reported by Savitz (1978) for bass of approximately 50 g or less, but less than those reported by Cochran and Adelman (1982) for bass of roughly 100–300 g (sample sizes in their study, however, were much smaller than those used here). Our simulation of size-selective mortality resulted in apparent mean instantaneous rates of growth that were biased by 0.8–5.1 and 2.4–18.2% for bass distributions with initial mean body mass of 200 g and standard deviations of 5.33 and 17.77 g, respectively. These biases are much lower than the discrepancies of 41.5–58.8% revealed by Ricker's (1975) analysis of Hile's (1936) data for ciscoes (as *Leucichthys artedii*).

The use of growth of individually tagged fish in conjunction with bioenergetics models permits some potentially useful and interesting analyses. For example, this sort of data provides a basis for comparison with growth rates derived from changes in mean body mass. In addition, it becomes possible to place "confidence intervals" around estimates of mean *P* values (such intervals are probably at best minimal estimates because they do not take into account such factors as dietary variability among individual fish) and to make at least quasi-statistical comparisons of food consumption rates; similar comparisons only recently became possible with more direct field estimates of food consumption (Worobec 1984; Pennington 1985). That food consumption varies with body mass may necessitate treatment of body mass as a covariate during analyses of *P* values or food consumption rates (as above). Indeed, the relationship between body mass of individual fish and *P* value (as a measure of foraging success) may be of primary interest if, for example, fish of a limited size range are expected to benefit most heavily from a particular management scheme or experimental manipulation.

We have shown that error due to use of change in mean body mass is miniscule. Biases in estimates of change in mean body mass may introduce significant error into estimates of food consumption, but energetics models in general appear to be relatively insensitive to these biases. The results of this study and earlier efforts (Cochran and Rice 1982; Rice and Cochran 1984) should engender greater confidence in the coupling of energetics modeling with appropriate field measures to replace more labor-intensive methods of estimating food consumption rates.

We do not claim to have exhausted the factors that might potentially lead to biased estimates of food consumption from changes in mean body mass. Rather, we suggest that researchers using energetics models can routinely and easily perform analyses of the type described herein under simulated conditions most appropriate to their particular studies. These analyses may include consideration of such factors as temperature, *P* value, sampling interval, and asymmetry in distributions of initial body mass.

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LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

Some Remarks on "Functional Maturity and Terminal Molt of Male Snow Crab, *Chionoecetes opilio*" by Conan and Comeau

According to Conan and Comeau (1986), the molt to morphometric maturity is identified as final for male snow crabs (*Chionoecetes opilio*) (Majidae). Their use of the literature on other species of Majidae to support this conclusion implies that other male *Chionoecetes* also fail to molt following puberty. If true, this would be a significant advance in the understanding of Tanner (snow) crab life history and, as discussed by the authors, would have significant impacts on present management strategies.

However, our review of the literature, examination of the data presented in Conan and Comeau's paper, and our analysis of other data on *C. opilio* and *C. bairdi* collected by the Alaska Department of Fish and Game and by the National Marine Fisheries Service lead us to conclude that *C. opilio* and *C. bairdi* do molt after puberty, although perhaps at a reduced frequency. In addition, we did not find that the literature strongly supported Conan and Comeau's implication of an absence of postpuberty molting throughout the genus.

Literature Support

Conan and Comeau primarily support the terminal molt conclusion using a recognized standard reference for majid crabs by Hartnoll (1963) who described the biology of 14 species of majid crabs found near the Isle of Man. Hartnoll stated, based on his own studies and those of Teissier (1935) and Vernet-Cornubert (1958), that "the molt of puberty is the last molt; this has previously been demonstrated for *Pisa tetraodon* and *Maja squinado*, and it is similarly the case with the species studied in the Isle of Man." Hartnoll did not present as definitive his statement "maturity molt equals terminal molt," as indicated by an additional statement: "It is, then, almost certain that there is no further molting after the molt of puberty." Items of evidence presented to support this conclusion are (1) the size of encrusting organisms, (2) telltale signs of molting, and (3) aquarium observations of molting for pre- and postpuberty crabs. While these items certainly suggest a cessation of molting for postpuberty crabs, the items are not definitive and may reflect an increase in the intermolt period with the achievement of maturity.

Study Results

Conan and Comeau were able to identify mature males by the use of principle component analysis, followed by linear discriminant function analysis, and check for the occurrence of molting males by aquarium and field experiments using essentially the same methodology as the three previously cited works.

Again, while Conan and Comeau's results suggest a terminal molt with maturity, the results are not definitive, as suggested by the following statements (p. 1716):

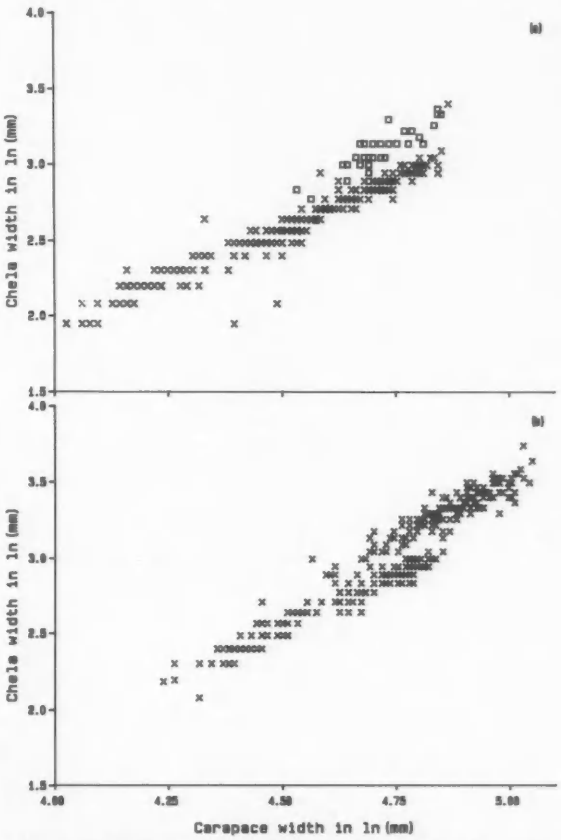

FIG. 1. Allometric relationship between chela width and carapace width for male *Chionoecetes bairdi* mature mating crabs (□, $N = 38$) and growth study crabs (×, $N = 318$) (a) prior to molt and (b) following molt.

(1) "Of the 110 morphometrically mature males maintained in the aquarium from November to September, none molted." Since the males were not in captivity when they molted to maturity, it seems impossible to conclude that prior to capture they had not undergone additional molts after their puberty molt. It is possible that the intermolt period for mature *C. opilio* may exceed 10 mo. In a study of *C. bairdi* growth, the intermolt period for mature males was estimated to be approximately 18 mo (Donaldson et al. 1981).

(2) "Of 260 morphometrically mature males sampled from the catch in *Nephrops* trawls from July to December...no individuals were ever found in the premolt 'D' or molt 'A' stage." Conan and Comeau's sampling effort occurred over a maximum of 6 mo. The authors do not address the length of the molting period for *C. opilio*. For *C. bairdi*, it is our personal observation that the premolt and molt stages are very short and

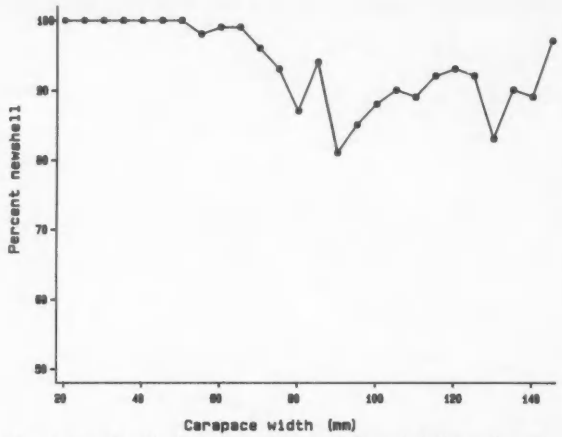

FIG. 2. Relationship between carapace width and shell age for male *Chionoecetes opilio* collected during the 1975 National Marine Fisheries Service Bering Sea trawl survey ($N = 4443$).

an intense effort over a period of time is necessary to locate these individuals. If the two species are similar, then the temporal and spatial sampling considerations should have been addressed. Without these the significance of the trawl data is obscure. We suggest that additional sampling during January through June and a larger sample size would be necessary to observe the existence of terminal molting.

New and Uncited Data

We repeated the procedures of Conan and Comeau using male *C. bairdi* data ($N = 318$) from a growth study (Donaldson et al. 1980) along with data from a study on 38 mating crabs (W. E. Donaldson, unpubl. data). The natural logarithm of chela width and the natural logarithm of carapace width were used as the two variables. The mating crabs were mature males as verified by successful fertilization of primiparous female egg clutches. The crabs from the growth study were thought to be primarily immature. The difference in the allometric relationship supported the assumption (Fig. 1a). To determine if the two groups were significantly different, a linear discriminant function was calculated. A significant difference ($P < 0.001$) did exist and the function provided a 98% correct classification. Three of the mating crabs were classified as immature, suggesting that some mature animals may not be discernible from immature crabs based on morphometry alone. Five of the immature animals were classified as morphometrically mature and three of the crabs had a very high probability of belonging to the mature group (posterior probability = 1.000, 0.998, 0.998, 0.868, 0.691). All of the crabs in the growth study subsequently molted in holding pens, including those classified as morphometrically mature. This indicates that there was a molt to maturity for a portion of the crabs in the "immature" group (Fig. 1b). The results show that several crabs were morphometrically mature prior to molting and subsequently molted successfully.

In a study by Adams and Paul (1983), *C. bairdi* male crabs of 65–75 mm carapace width mated. These mating male crabs were morphometrically immature. According to Colgate and Hicks (1983), crabs of this size and one cohort group larger are almost always newshell animals, suggesting that they are not anecdysic. Additional support for molting after maturity is evident in a study involving the ageing of barnacles found on *C. bairdi* (Paul and Paul 1986). Eighty-seven percent of the terminal molt female crabs from Cook Inlet, Alaska, were carrying barnacles, and 42% of the female crabs had barnacles that had survived three or four growing seasons. However, none of the 1180 males examined carried barnacles three or more growing seasons old. The males ranged in size from 140 to 190 mm carapace width (mature animals). If mature males terminal molted as do females at the molt to maturity, we would expect the age composition of settling barnacles to be similar between the sexes of *C. bairdi*. Also, Tester and Carey (1986), in a study of *C. tanneri*, stated, "Observations on general carapace condition and abundance of epifauna indicate that adult male *C. tanneri* do molt frequently enough to maintain their carapaces relatively free of epifauna and lesions caused by bacterial infection."

Observations on the shell age of the Bering Sea male *C. opilio* population do not support a terminal molt at maturity conclusion for that population. Figure 2 depicts the shell age of male *C. opilio* captured during the 1975 National Marine Fisheries Service research survey (R. Otto, Box 1638, Kodiak, AK 99615, unpubl. data). We present the 1975 survey year data for the area south of 58° latitude because *C. opilio* was not the target species, and therefore, a fishing-down effect had not occurred. The size at which 50% of male *C. opilio* are mature is estimated to be at 65-mm carapace width (Somerton 1981). If the molt to maturity is a terminal molt, and in the absence of heavy exploitation, we would expect to observe a large accumulation of oldshell males in the mature portion of the population. As Fig. 2 depicts, this was not the case. The majority of large crabs were newshell (recently molted) animals. The oldshell crabs that were present most likely represented a decrease in growth rate (molting frequency) with the achievement of sexual maturity.

Conclusions

We feel that Conan and Comeau's conclusions are overstated based on our review of their supportive literature and their field studies. In addition, we show that *C. bairdi* do molt after the molt to morphometric maturity. We cite additional information on the species *Chionoecetes* that is in contrast with the findings of both Hartnoll (1963) and Conan and Comeau. These data suggest that mature male *Chionoecetes* do not terminally molt at the molt to maturity. In a management framework it is important to resolve the terminal molt at maturity issue, primarily because probabilities of these molts occurring as a function of size or sexual maturity are important to the establishment of legal size limits — William E. Donaldson and B. Alan Johnson, *Alaska Department of Fish and Game, Division of Commercial Fisheries, 211 Mission Road, Kodiak, AK 99615, USA.*

(J9298)

Acknowledgements

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Reply to Donaldson and Johnson

Conan and Comeau (1986) introduced the concept that male *Chionoecetes opilio* molted for the last time in their life span at onset of "morphometric maturity." During this "terminal molt," their chelae differentiated to a new shape specialized for holding the female during a precopulatory embrace. It was previously believed that only female *Chionoecetes* spp. reached a terminal molt at onset of morphometric maturity. Morphometric maturity of the females is identified by a differentiation of the shape of the abdomen for holding the brood, and less markedly, and not functionally as in the male, by a differentiation of the shape of the chelae. The males were thought to keep on growing after functional maturity, and this was said to "explain" the considerable size discrepancies observed between adult males and adult females.

The "morphometric maturity" of males should not necessarily be equated with functional maturity, i.e. the aptitude to mate successfully. We have never observed so far mating of morphometrically immature males with hard-shell (intermolt) females, either in aquaria or in the wild. However, spermatophores can be found in the genital tract of all morphometrically immature males larger than 40 mm carapace width. Watson's (1970, 1972) work would suggest that such males may have the aptitude to mate with soft-shell females shortly after the latter achieve the terminal molt.

Donaldson and Johnson report that (1) the analysis of their own data would indicate that *C. opilio* and *C. bairdi* do molt after puberty, eventually at a reduced frequency, and (2) the literature does not strongly support Conan and Comeau's (1986) interpretation of *Chionoecetes* growth. We shall discuss their opinion in an evolutionary and biogeographical context and analyse the evidence presented.

Available Literature

Through an extensive review of British, Canadian, French, Japanese, Soviet, and U.S. literature, G. Y. Conan and R. W. Elner (in prep.) retraced the genesis of the opinion that only females, and not the males, reached a terminal molt at onset of morphometric maturity in the genus *Chionoecetes*. Cross references in the literature lead Conan and Elner to a provisional statement made by Yoshida (1941), who suggested that the size disparity he observed between sexes might be explained by an interruption of the growth of the females at maturity.

Yoshida (1941) observed that the females incubated eggs under their abdomen all year round and would spawn again immediately after hatching of the previous brood; he concluded that females would not normally molt after maturity at the expense of loosing their brood. Yoshida did not actually observe that morphometrically mature males would molt and grow; nevertheless, this latter opinion was frequently cited, and later became a dogma. Conversely, Conan and Elner noted that, for all other genera of the same family of majid crabs which had been objectively studied, the literature always reported a terminal molt at the onset of morphometric maturity, both for males and females.

Biogeography and Evolution

Life histories in *Chionoecetes* populations studied by Donaldson and Johnson, and by us, may differ. The genus *Chionoecetes* is circumarctic (Rathbun 1893), but the continuity of Atlantic and Pacific populations is not documented. The depth distributions of *C. opilio* differ in the Atlantic and Pacific oceans, and other ecological characteristics may also vary. The species *C. bairdi*, on which Donaldson and Johnson report, is not known in the Atlantic. The growth patterns of crustaceans may vary considerably among geographic populations and among species (Conan 1985).

The hypothesis that some individuals in certain populations of *Chionoecetes* may have retained the ability to grow after maturity as an archaic character is possible. The chelae of *Chionoecetes* do not differentiate as markedly at onset of the terminal molt as in other genera of Majidae such as *Pisa*, described by Vernet-Cornubert (1958); this may suggest that *Chionoecetes* have not yet fully made the choice for a terminal molt as other genera of majids.

The incidence of individuals bearing archaic characters may vary between populations. Fishery management must be based on life history patterns common to most individuals harvested, not on patterns specific to exceptional individuals. We shall attempt to assess the incidence of possible cases of molt after morphometric maturity in Pacific and Atlantic populations of *Chionoecetes*.

Pacific Chionoecetes

Experimental evidence — In order to demonstrate molting after morphometric maturity in Pacific *Chionoecetes*, Donaldson and Johnson use two independent sets of data for *C. bairdi*: (1) 318 males which molted with success in aquaria and (2) 38 males which mated with success in aquaria. It should be stressed that the individuals which mated were not the same ones as those which were observed to molt in a separate experiment.

They use the same morphometric measurements and the same discriminant analysis as Conan and Comeau (1986). They contrast the data from premolt individuals in the growth experiment with the data from mating individuals in the mating experiment and calibrate a discriminant function. The discriminant function is found to be 98% efficient for distinguishing the two sets of data, i.e. the two swarms of points slightly overlap. This implies that about 2% of the observations will be misclassified

in the sets used for calibration, as well as in similar data to be assessed, even if the assumptions of binormality and equality of matrices of variance covariance are respected.

Donaldson and Johnson identify 5 individuals out of 318 from the swarm of molting individuals that are classified by the discriminant function as pertaining to the swarm of morphometrically mature individuals. They conclude that these individuals molted after having reached the characteristics of morphometric maturity.

Our comments refer to the statistical analysis and to the biological definition of maturity:

(1) In terms of statistical analysis, a discriminant function should not be used for reclassifying the observations which were used for calibrating its parameters. The discriminant function attributes any given single observation to either set on the basis of the highest of two nonmutually exclusive probabilities calculated for this observation only. The probability that the 5 most extreme observations out of 318 do not pertain to the same set as the other 313 should be calculated globally, given that there are 318 replicates. For instance, a 98% efficiency implies that some 6 observations out of 300 may be expected to be misclassified.

It would be risky to draw conclusions even if the amount of stray points was significantly greater than expected by the overlap of standard binormal ellipsoids. A few points at the limit of significance may simply reveal slight departures from the assumptions required by the methodology. For instance, a deformation of the chela occurs in rare cases when the ecdysis is abnormal. Morphometrically mature males may bear a small claw if regeneration of a missing claw was not fully achieved prior to the terminal molt.

(2) In terms of the biological definition of maturity, we do not recommend using sets of data of independent origin for contrasting morphometrically immature and morphometrically mature individuals. We have observed differences in the parameters of discriminant functions calculated for distinct localities.

We do not recommend using as criteria for morphometric maturity the fact that individuals successfully mated. If the females used for testing the males were hard-shell multiparous females, the success of their brood may have resulted from an earlier mating, since sperm may remain active in the female spermatheca for up to 2 yr (Paul 1984). If the females used were soft-shell primiparous females having recently achieved the terminal molt, we do not exclude the possibility of mating with morphometrically immature males. In our observations both in aquaria and in the wild, only morphometrically mature males mated with females in hard-shell and terminal molt. Nevertheless, males bear mature gonads producing spermatophores long before they reach the terminal molt. We do not reject the possibility, as suggested by Watson's (1970) observations, that such individuals may have the ability to mate with soft-shell females shortly after the latter achieve their terminal molt.

Circumstancial evidence — Donaldson and Johnson provide further arguments for molt after morphometric maturity, based on frequency of old-shell individuals among large males and females. They mention that the percentage of old shells is always greater among large females than among large males and that there is no accumulation of old-shell males among the mature portion of the population. We believe that this does not necessarily imply that males grow after morphometric maturity. The presence of new shells among large males is explained by (1) the wide overlap of size frequency distributions of morphometrically immature vs morphometrically mature individuals, as shown by their own data (their fig. 1a), because morphometric maturity is reached over a wide range of sizes (their fig. 1b), and (2) the fact that mortality may be higher among males than among females, particularly when a fishery selectively harvests large individuals, the males being much larger than the females.

Conclusions on Pacific Chionoecetes — The possibility of molting after morphometric differentiation of the claw in *C. bairdi* would be most interesting in terms of evolution of the majids, but the facts do not appear conclusive. We would suggest an experimental approach.

A large sample of male *C. bairdi* should be sorted out by multivariate or by a simple bivariate analysis according to the morphometry of the claw. A discriminant function should be fitted by contrasting morphometrically immature and mature sets previously sorted out. About 100 individuals classified as morphometrically mature by the discriminant function and beyond the range of 99% of the immature distribution should be kept in aquaria through the molt season and observed for preparation to molt by reading molt stages on the maxillipeds, as described by Moriyasu and Mallet (1986). About the same number of individuals of equivalent sizes, but classified as morphometrically immature, should be maintained under similar conditions and would be used as controls for incidence of molting.

Gulf of St. Lawrence Chionoecetes opilio

Since Conan and Comeau (1986), we have obtained further information on Gulf of St. Lawrence *C. opilio* molting. Molting of large immature males is limited to one well-marked period in the spring. Seasonality of molting is a usual phenomenon among Crustacea (Conan 1985). During July to September 1987 trawl surveys, we never observed premolt individuals among 6081 mature and 2004 immature males examined (M. Moriyasu, pers. obs.). From October to November, 312 morphometrically mature and 208 immature males caught by trawling were examined for molt stages. No morphometrically mature males were found in the premolt stage, while 50 immature males were found in premolt stage D_0.

Ninety four immature males (30 individuals initially in D_0 and 64 in C_4) survived more than 6 months in aquaria at temperatures simulating their natural habitat. All of these advanced in molt stages during the winter and molted successfully or died in the process of molting between the following late March and late April. No ecdysis was ever observed to take place outside of this period. Among 141 morphometrically adult males kept in aquaria under identical conditions, none molted or were ever found in the premolt stage (M. Moriyasu, pers. obs.).

It seems that the difficulty in encountering molting individuals of *Chionoecetes* spp. mentioned by Donaldson and Johnson was formerly mainly due to (1) the difficulty of identifying premolt stages such as D_0, (2) the use of inappropriate sampling tools (trawls that do not dig sufficiently well in the sediment, or traps in which molting individuals do not enter), and (3) fishing outside the molt season rather than fishing in inappropriate locations.

Further information is provided by radioimmunoassay of the molt hormones (ecdysones) within the hemolymph of sexually mature and immature snow crabs (Cormier 1986). Among crabs in intermolt C_4, morphometrically mature individuals have very little ecdysone circulating in the hemolymph in comparison with

immature individuals of similar sizes. Low levels of ecdysones may be expected if male snow crabs stop molting after the molt to morphometric maturity.

We believe that these facts strongly support Conan and Comeau's (1986) earlier data and interpretation of growth of male snow crabs.

Conclusions

Although we cannot sample all year round in the Gulf of St. Lawrence due to a thick ice coverage from December to March, we believe that there is by now sufficient evidence for ascertaining that molting after morphometric maturity occurs among male *C. opilio* with an extremely low, if not a null, incidence. The absence of premolt stages among morphometrically mature individuals cannot be explained solely by an increase in time interval between successive molts, as a function of size. (1) For instance, a time interval of 18 mo between molts suggested by Donaldson and Johnson would only imply that some 63% instead of 100% of morphometrically mature males would be found in premolt stages at the beginning of the molt period. (2) Further, premolt stages are common among large morphometrically immature males but are never found among morphometrically mature males of the same size.

It is impossible to demonstrate statistically that molting never occurs after morphometric maturity because the power of the discriminant function used for identifying morphometrically mature individuals does not usually exceed 99%, the function will therefore misclassify about 1% of the individuals. Given that no individuals in premolt stage were found ($X = 0$), whatever the sample size (n), there will still be a 5% chance that the actual proportion of premolters is $P = 3/n$, as shown by the Poisson distribution. It is useless to oversample repeatedly thousands of individuals if a few hundred provide a sufficient precision, on the estimates of the frequency of occurrence of molting individuals, for practical purposes. Rather, one should concentrate on sampling at the right time of the year or in the right location.

It is not yet possible to reject the possibility that molting of morphometrically mature individuals would be phased up and occur cyclically with a periodicity of several years. This type of life history would be, however, quite extraordinary as an adaptative strategy and does not seem realistic.

It is not possible to generalize our observations to other populations of *Chionoecetes* because data are not available. However, we feel that there is presently no available evidence for any population of any species of *Chionoecetes* that molting occurs after morphometric maturity with frequencies higher than what may be expected from teratology. In the southern Gulf of St. Lawrence, the occurrence of such individuals over the past years has been undetected.

The existance of a terminal molt occurring over a wide range of sizes, including below the minimal legally harvestable size, cannot be ignored for fisheries management purposes and should motivate revised management approaches, as acknowledged by Donaldson and Johnson — G. Y. Conan, M. Comeau, M. Moriyasu, and R. Cormier, *Department of Fisheries and Oceans, Gulf of St. Lawrence Region, Moncton, N.B. E1A 3E9.*

(J9444)

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 43, No. 10 — article by T. A. Jackson: *Methyl mercury levels in a polluted prairie river–lake system: seasonal and site-specific variations, and the dominant influence of trophic conditions.*
Page 1874, right column: Line 11 should read "a dry subhumid to semiarid..."
Page 1876, left column: Line 2 of text should read "conductivity following acidification on an..."
Page 1884, left column: Line 18 should read ... may well be due to the..."

VOLUME 44, No. 11 — article by L. M. Dickie and P. R. Boudreau: *Comparison of acoustic reflections from spherical objects and fish using a dual-beam echosounder.*
Page 1915, right column: Line 6 should read "(0.03-m) pulses generated..."
Page 1917: Equation (10) should read "$10 \log E_w^2 = \text{constant} + a(10 \log E_n^2)$"

VOLUME 44, No. 11 — article by R. C. Chambers and W. C. Leggett. *Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder* (Pseudopleuronectes americanus) *with a review of variation in other species.*
Page 1939, right column: The first line of the equation defining $\sigma_{CV_1'-CV_2'}$ should read

$$\sigma_{CV_1'-CV_2'} = \{(1 + 1/4n)^2(1/2n)$$

Page 1942, Table 2: The asterisks denoting levels of statistical significance were omitted from the last column (following t values). The table should show that all t values were statistically significant at the $p < 0.001$ level except for starry flounder, population 6, where $p < 0.01$.

VOLUME 44, Suppl. 2 — *International Symposium on Stocks Assessment and Yield Prediction (ASPY).*
The Journal offers sincere apologies for two unfortunate oversights that occurred in this Supplement.

Masthead: Dr. P. E. K. Symons should be credited as the Special Scientific Editor for ASPY.
Page 501: The following individuals (with their areas of responsibility) should be added as:

ASPY Coordinators

R. J. H. Beverton (*Overseas*)
P. J. Colby (*N. American Inland*)
L. M. Dickie (*N. American East Coast*)
B. E. Skud (*N. American West Coast*)

VOLUME 44, Suppl. 2 — article by L. M. Dickie, S. R. Kerr, and P. Schwinghamer: *An ecological approach to fisheries assessment.*
Page 71: Table 1 column headings: units for body size should be kcal; there should be no units for the *P/B* columns.

VOLUME 45, No. 1 — article by T. A. Jackson: *The mercury problem in recently formed reservoirs of northern Manitoba (Canada): effects of impoundment and other factors on the production of methyl mercury by microorganisms in sediments.*
Page 101: Table 4 caption should contain the following: — = no sample.
Page 110, right column: Line 15 should read "...whether there are any local..."
Page 114, Table 17: Line 1 of caption should read "...sediment CH_3Hg^+ produced..."; column 6, row 9: the regression coefficient for CH_3Hg^+ produced, all sites sampled in 1981, and Org. C in June 1981 sediment should be positive, i.e. 0.605; rows 13 and 14 of column 1 should read "A_{465nm} of NaOH extract..."
Page 115, Fig. 9: "CM" on the figure should read "CML."

VOLUME 45, No. 6 — Article by P. E. Ihssen, J. M. Casselman, G. W. Martin, and R. B. Phillips: *Biochemical genetic differentiation of lake trout* (Salvelinus namaycush) *stocks of the Great Lakes region.*
Page 1023, left column: Line 2 should read "use, such as 2% for Atlantic cod (*Gadus morhua*) (Mork et al.".

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Mosegaard, H., H. Svedäng, and K. Taberman. Uncoupling of somatic and otolithic growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response

Andersson, T., L. Förlin, J. Härdig, and Å. Larsson. Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents

Jonsson, B., S. Skúlason, S. S. Snorrason, O. T. Sandlund, H. J. Malmquist, P. M. Jónasson, R. Gydems, and T. Lindem. Life history variation of polymorphic Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland

Corti, M., R. S. Thorpe, L. Sola, V. Sbordoni, and S. Cataudella. Multivariate morphometrics in aquaculture: a case study of six stocks of the common carp (*Cyprinus carpio*) from Italy

Vandevelde, T., L. Legendre, S. Demers, and J.-C. Therriault. Interrelationship between in vivo fluorescence of phytoplankton and light beam transmission with reference to fluorescence yield

Wissmar, R. C., and C. A. Simenstad. Energetic constraints of juvenile chum salmon (*Oncorhynchus keta*) migrating in estuaries

Bergman, H. L., J. S. Mattice, and D. J. A. Brown. Lake Acidification Fisheries Project: adult brook trout (*Salvelinus fontinalis*)

Booth, C. E., D. G. McDonald, B. P. Simons, and C. M. Wood. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*)

Wood, C. M., R. C. Playle, B. P. Simons, G. G. Goss, and D. G. McDonald. Blood gases, acid–base status, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure

Wood, C. M., D. G. McDonald, C. E. Booth, B. P. Simons, C. G. Ingersoll, and H. L. Bergman. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes

Wood, C. M., B. P. Simons, D. R. Mount, and H. L. Bergman. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 2. Blood parameters by cannulation

McDonald, D. G., and C. L. Milligan. Sodium transport in the brook trout, *Salvelinus fontinalis*: effects of prolonged low pH exposure in the presence and absence of aluminum

Walker, R. L., C. M. Wood, and H. L. Bergman. Effects of low pH and aluminum on ventilation in the brook trout (*Salvelinus fontinalis*)

Mount, D. R., C. G. Ingersoll, D. D. Gulley, J. D. Fernandez, T. W. LaPoint, and H. L. Bergman. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity, and progeny survival

Mount, D. R., J. R. Hockett, and W. A. Gern. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 2. Vitellogenesis and osmoregulation

Tietge, J. E., R. D. Johnson, and H. L. Bergman. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum

Notes

Rowe, L., J. Hudson, and M. Berrill. Hatching success of mayfly eggs at low pH

Hiltz, K. S., J. G. Dean, P. Schwinghamer, and D. J. Belliveau. Computer-driven motor control with positional memory for mechanical microscope stage

Perspectives

Miller, T. J., L. B. Crowder, J. A. Rice, and E. Marschall. Larval size and recruitment mechanisms in fishes: toward a conceptual framework

Turkey Lakes Watershed Study

Kelso, J. R. M. Preface

Jeffries, D. S., J. R. M. Kelso, and I. K. Morrison. Physical, chemical, and biological characteristics of the Turkey Lakes Watershed, central Ontario, Canada

Sirois, A., and R. J. Vet. Detailed analysis of sulphate and nitrate atmospheric deposition estimates at the Turkey Lakes Watershed

Vet, R. J., A. Sirois, D. S. Jeffries, R. G. Semkin, N. W. Foster, P. Hazlett, and C. H. Chan. Comparison of bulk, wet-only, and wet-plus-dry deposition measurements at the Turkey Lakes Watershed

Semkin, R. G., and D. S. Jeffries. Chemistry of atmospheric deposition, the snowpack, and snowmelt in the Turkey Lakes Watershed

Jeffries, D. S., R. G. Semkin, R. Neureuther, and M. Seymour. Ion mass budgets for lakes in the Turkey Lakes Watershed, June 1981–May 1983

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